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## Thesis objectives and structure

The major objective of this thesis was to investigate the historical and recurrent processes shaping the genetic make-up of *Fucus ceranoides*, an estuarine seaweed lacking planktonic developmental stages. To this end, a range of molecular markers was used to describe the population genetic structure of this seaweed throughout its entire range and at more regional spatial scales. Specifically, the modern patterns of genetic diversity and differentiation of *F. ceranoides* were used to test the expected structuring effects of its particular life-history (e.g. habitat discontinuity, restricted dispersal), to infer its patterns of population genetic connectivity at large and small spatial scales, to infer the extent of introgression with related species, and to reconstruct its historical biogeography in the Northeast Atlantic.

CHAPTER I provides a general introduction to population genetics theory, with special emphasis on the factors that interact to shape the population structure of marine organisms. The specificity of large brown seaweeds in the context of marine phylogeography is discussed, and some interesting findings highlighted. Finally, the biology of the species investigated in this study is presented.

In CHAPTER II a combination of sequence markers is employed to resolve the phylogenetic relationships and the patterns of genetic exchange between *F. ceranoides* and two parapatric congeners. Because introgression was apparent for cytoplasmatic markers, the mtDNA phylogeography of *F. ceranoides* is also described to further investigate the geographical scope of introgression and its relationship with the species demographic history.

In CHAPTER III, the population genetic structure of *F. ceranoides* is examined throughout its entire distributional range with microsatellite markers. The objective was to reconstruct the historical biogeography of *F. ceranoides* and to investigate the effects of

restricted dispersal and demographic history on the geographical organization of its genetic variation.

In CHAPTER IV, the fine-scale phylogeographic structure of *F. ceranoides* in NW Iberia is described using both microsatellites and mtDNA sequence data. In the previous chapters three distinct phylogroups were found in this very restricted shoreline. Here, the historical and recurrent processes contributing to generate (and maintain) these high levels of regional population subdivision (in the absence of obvious dispersal barriers) are investigated.

Finally, CHAPTER V provides a synthesis of the results of the preceding chapters and highlights the main contributions of this work.

# **CHAPTER I.** GENERAL INTRODUCTION

## Marine population genetic structure

Most, if not all marine species, exhibit some degree of population genetic structure, i.e., their modern populations differ to some extent in their genetic composition. A complete lack of differentiation across a species range (i.e., all populations having the same allele frequencies) would only be possible if the entire species was constituted by a single, very large group of randomly mating individuals. From a variety of reasons (biological, behavioural, geographical, ecological and geological), even the most mobile species are normally far from being panmictic throughout their entire ranges. As a result, populations inevitably diverge in time and space to some extent.

Populations evolve by changes in the frequencies of alleles, the alternative forms of genes that constitute heritable diversity among conspecific individuals. Allele frequency change results from the interaction of distinct evolutionary forces: *mutation* (or introgression) of genes, which introduces new allelic variants in the populations; *genetic drift*, the stochastic fluctuation of allele frequencies resulting from the inter-generational sub-sampling of alleles in finite populations; *gene flow*, the exchange of genes between populations resulting from effective migration; and *selection*, the deterministic increase (and purge) of gene variants due to fitness effects (increased/reduced survival or reproductive output) (Hellberg *et al.* 2002). Over sufficiently long periods of time, and in the absence of demographical change, the genetic differentiation of populations will arrive at a state of equilibrium determined by the balance among these opposing forces. Mutation, drift and diversifying selection will cause populations to diverge, whereas migration and stabilizing selection will act to genetically

homogenise populations and maintain the genetic cohesion of a biological species (Slatkin 1987).

The theoretical effect of gene-flow on allele-frequency divergence is the conceptual link between marine connectivity and population genetics (Hedgecock *et al.* 2007). If equal-sized populations are in equilibrium (i.e. the introduction on new alleles within populations from gene-flow and mutation is in equilibrium with the loss of alleles via genetic drift), the extent of neutral genetic differentiation between them will reflect their average rate of gene flow. In the Island Model (Wright 1931) at equilibrium, the absolute number of migrants exchanged each generation can be estimated as $Nm = (1– FST)/ (4FST)$, where $N$ is the effective size of the local population and $m$ is the proportion of migrants entering that population each generation.

Patterns of genetic differentiation can nevertheless represent poor proxies for modern population connectivity, because in natural populations the equilibrium assumption is often violated [see (Marko & Hart 2011b)]. The conditions that generate any particular distribution and subdivision change over ecological and evolutionary time scales, and it takes time for populations to progressively approach new genetic equilibriums [e.g. (Richmond *et al.* 2009)]. The number of generations required depends on the effective population sizes and rates of gene-flow (Palumbi 2003; Hedgecock *et al.* 2007; Hellberg 2009; Marko & Hart 2011a), but can be substantial. For species characterized by relatively large effective population sizes and/or low migration rates it may mean several thousands of generations and thus inferred patterns of connectivity will often be severely confounded by the vestiges of history (Benzie 1999; Hellberg *et al.* 2002; Poissant *et al.* 2005; Teske *et al.* 2011). On the

positive side, they typically allow the examination of historical patterns of population stability, vicariance and colonization with finer resolution (Hewitt 2000; Pelc *et al.* 2009).

Marine biologists have long been interested in interpreting the geographic patterns of genetic variation and investigate the importance of several interacting factors that may be potentially responsible for them. Among others (e.g. mating systems, selection, introgression), the most important drivers of population genetic structure are the specie's intrinsic dispersal potential, population subdivision and population history.

*Dispersal potential*

Dispersal, the moviment of individuals or their propagules across space, has profound ecologic and evolutionary consequences. It allows the connectivity of populations in heterogeneous landscapes, the persistence of regional meta-populations (despite local extinctions), and the tracking of favourable environmental conditions in an ever changing world. As such, the patterns of dispersal have wide-ranging ramifications for the population dynamics, genetic structure, cohesion, resilience, distribution and evolution of species. The spatial scales where dispersal is important may vary considerably depending on the process of interest. When referring to population connectivity, the level of exchange must be sufficient to impact the demographic properties of the local population(s) (Johnson *et al.* 2009; Lowe & Allendorf 2010). Such ecologically relevant exchange rates are often several orders of magnitude larger than the levels required to influence the genetic connectivity of populations via gene-flow, which often extends into the narrower tail of a species dispersal kernel. In the other extreme, extreme events of long-distance dispersal may be insignificant for the genetic

connectivity of very distant populations, but still allow the colonization of vacant habitat patches.

Historically, marine populations have been considered to be relatively open, with populations demographically and genetically connected over broad spatial scales. On one hand, the dense and fluid characteristics of the marine environment and the speed of ocean currents create the possibility for extremely long-distance dispersal. Life-history characteristics of most marine organisms (e.g. high fecundity and planktonic larvae) and empirical observations that local recruitment and local production are often largely decoupled, also argued that marine systems in general were connected over larger spatial scales than terrestrial systems. Accumulated evidence, however, has been revealing a much more complex picture of marine dispersal, showing that even in the sea there is a wide continuum in dispersal potential (Mora & Sale 2002; Kinlan & Gaines 2003; Kinlan *et al.* 2005; Bradbury *et al.* 2008).

In part this variation is not unexpected because marine organisms (as terrestrial ones) display an incredible diversity of life-forms, life-cycles, life-history traits, habitats and ecologies. In most benthic/sessile coastal species, gene flow is mostly mediated by early life-history stages, namely by minute planktonic larvae. The dispersal potential of these organisms is normally high, but nevertheless very variable. Some characteristics of larvae, such as their longevity (time spent in water column) and behaviour, can influence (and provide relatively good proxies of) the spatial scale and magnitude of dispersal and hence population subdivision [(Bohonak 1999; Siegel *et al.* 2003), but see (Riginos *et al.* 2011)]. Other coastal organisms are sessile and lack such planktonic dispersive stages (e.g. seaweeds and direct-developing invertebrates), or disperse both as larvae and adults (e.g. coastal pelagic fish). In between these extremes, which are expected to be either very poor or very good dispersers

(and therefore highly or very loosely structured), lies a vast range of dispersal strategies and spatial scales of demographic and genetic connectivity.

*Population subdivision*

The habitat specificities of species can also influence their intrinsic dispersal potential. For instance, species inhabiting highly discontinuous habitats (e.g. estuaries) are prone to exhibit marked population genetic structure because of their inherent geographic subdivision (Bilton *et al.* 2002). Distance *per se* is among one of the most pervasive factors driving population genetic structure of marine organisms. Because the geographical distribution of a species typically exceeds by many orders of magnitude the dispersal range of individuals, dispersal (and gene-flow) between populations often decreases with increasing distance. As a result of this tendency, populations that live near each other may tend to be genetically more similar than populations that live further apart. In other words, marine populations may be genetically differentiated through isolation by distance [IBD; (Slatkin 1993; Hutchison & Templeton 1999; Hellberg *et al.* 2002; Palumbi 2003)]. Depending on the dispersal potential of a species, a pattern of IBD is expected to develop within certain spatial scales, but not others (Hedgecock *et al.* 2007).

More contingent features such as the regional distribution of available habitat (patchy versus continuous), local oceanographical or ecological barriers to dispersal and large habitat discontinuities can severely depress the realized scale of dispersal among regional populations (Sponaugle *et al.* 2002; Galarza *et al.* 2009; Plouviez *et al.* 2009). For instance, dispersal of pelagic larvae of the crab *Armases rubripes* across the estuarine plume of Rio de la Plata (Uruguay) is stalled because salinity and temperature are suboptimal for larval survival (Luppi *et al.* 2003). Similarly, the mantis shrimp *Haptosquilla pulchella* disperses across

thousands of kilometres of semi-contiguous coastlines, but gene-flow is apparently much reduced between populations separated by 300 km of open ocean (Barber *et al.* 2002).

*Population history*

The modern genetic structure of species reflects how evolutionary forces are currently acting within and between extant populations, but also how they have affected molecular variation in the past. In particular, the historical biogeography and demography of species are of special relevance (Marko 2004; Hickerson & Cunningham 2005). One recurrent pattern found in temperate terrestrial organisms (but also in the sea) is the contrasting levels of genetic diversity and structure between refugial and post-glacially recolonized regions (Hewitt 2000). Rather than meaning that high-latitude populations experience more gene flow than their southern counterparts, it reflects insufficient time for populations to achieve equilibrium. Similarly, phylogeographic breaks and contemporary oceanographic barriers (or biogeographical transition zones) are often mismatched in marine restricted dispersers (Pelc *et al.* 2009). Apparently, the historical patterns of vicariance and colonization in these organisms explain genetic structure better than modern (and typically more recent) patterns of gene-flow.

Historical, non-equilibrium situations include recent range expansions (Edmands 2001; Marko 2004; Fraser *et al.* 2009b), the rise (and fall) of dispersal barriers (Barber *et al.* 2000), meta-populations regulated by frequent local extinctions and (re)colonisations (Boileau *et al.* 1992; Whitlock 1992), and recent secondary contact of genetically differentiated lineages (Hobbs *et al.* 2009). In all these complex demographic situations, variations in effective population sizes, geographical range and time (since expansion, colonization, isolation or secondary contact) are also important factors underlying the distribution of modern genetic variation (in addition to rates of gene flow). The impact of population history

has perhaps been better appreciated by phylogeographers. Phylogeography is the field of study concerned with the principles and processes governing the geographic distributions of genealogic lineages (Avise 2000). Because DNA variation provides not only allele-frequency and geographic but also genealogic (evolutionary) information, it allows insight into how scored genetic variation evolved in both time and space. In this sense, sequence data generally perform better than frequency-based markers in teasing apart the effects of historical and modern patterns of connectivity.

Traditionally, phylogeography has been based upon the practice of gathering genetic data (normally mtDNA or cpDNA) from samples collected across a geographical range, reconstructing and interpreting the phylogenetic tree (or network) in a biogeographic context, and then looking for possible explanations (restricted gene flow, vicariance, range expansion, etc…) that could potentially have generated the observed patterns of genealogic/geographic association (Templeton *et al.* 1995; Avise 2004). With the full assimilation of the coalescent theory in newly available analysis (Kuhner 2009), this largely descriptive approach of seeking post-hoc explanations has been shifting to a more rigorous, statistical-based framework within which competing phylogeographic hypothesis can be specified and tested (Knowles & Maddison 2002; Marko & Hart 2011a).

**Phylogeography of large, canopy-forming brown macroalgae**

Large, canopy-forming brown algae (Heterokontophyta; Phaeophyceae) such as kelps (orders Laminariales and Tilopteridales) and fucoids (order Fucales) have several characteristics that make them potentially very attractive models to address important questions about marine connectivity, biogeography and conservation. They are fairly diverse (taxonomically and ecologically) and ubiquitous on many intertidal and shallow subtidal habitats throughout cold, temperate and tropical latitudes (Guiry & Guiry 2011). They are sessile, relatively large, and often easy to collect and preserve. Importantly, kelps and fucoids normally exhibit quite restricted dispersal when compared to fish and invertebrate species (Kinlan *et al.* 2005; Bradbury *et al.* 2008). This stems from the fact that their propagules (spores, gametes, zygotes) have planktonic periods of less than a day, and dispersal distances on the order of tens to few thousands of meters, i.e., of the same magnitude of seeds of many terrestrial plants (Santelices 1990).

Given their tipically small scales of dispersal, the members of this key, bio-engineering assemblage are expected to exhibit relatively "closed" populations when compared to most invertebrate and fish species, and therefore to provide alternative biological models to investigate the effects of dispersal, subdivision and history in the low end of the marine dispersal continuum. Given the long-standing interest in their biogeography (Van den Hoek 1975; South 1987; Luning 1990; Adey & Steneck 2001), phylogeny (Boo *et al.* 1999; Serrão *et al.* 1999; Yoon *et al.* 2001; Cho *et al.* 2006) and ecology (Hawkins & Hartnoll 1985; Chapman 1995), the wealth of information documenting the environmental basis of seaweed species distributions (Hoek 1982; Breeman 1988; Breeman 1990; Luning 1990), and the key structural role played by many species in shallow rocky-shore habitats (Steneck *et al.* 2002; Schiel & Foster 2006), large brown seaweeds would appear particularly attractive

phylogeographic models, but surprisingly they have remained until very recently among the most neglected groups in marine phylogeographic research (see Table 1.1; note that only one study had been published by 2007).

Recent studies confirm that large brown seaweeds exhibit unusually strong phylogeographic structures. The diverse genetic patterns already encountered (and spawning several biogeographic regions) have been successfully linked to a varied range of historical, biogeographic and demographic processes (see Table 1.1 for a synopsis), including the identification of glacial refugia and pathways of post-glacial colonization (Hoarau *et al.* 2007; Fraser *et al.* 2009b), patterns of inter-oceanic exchange (Coyer *et al.* 2010), modern and ancient vicariant processes (Fraser *et al.* 2009c; Cheang *et al.* 2010; Fraser *et al.* 2010) and the detection of cryptic species (Fraser *et al.* 2009a; Tellier *et al.* 2009; Tellier *et al.* 2011). The observation that the spread rates of several invasive seaweed species exceed their anticipated dispersal potential by several orders of magnitude (Kinlan & Gaines 2003; Kinlan & Hastings 2005), the significant post-glacial range expansions experienced by many species (Hoarau *et al.* 2007; Fraser *et al.* 2009b) and the historical colonization of remote islands (Hoek 1987) suggest nevertheless that secondary vectors of dispersal can effectively extend the expected dispersal ranges of some species by many orders of magnitude. Long-distance dispersal in seaweeds has long been suspected to occur, at least episodically, by means of drifting fertile fronds (Hoek 1987; Norton 1992; Hernandez-Carmona *et al.* 2006; McKenzie & Bellgrove 2008). Many species are naturally buoyant and if detached can be passively transported by surface currents. Dispersal via rafting probably constitutes an important mechanism assisting the establishment of new populations, but its relevance for the demographic and/or genetic connectivity of isolated adult seaweed populations can be much more modest (Fraser *et al.* 2009b; Fraser *et al.* 2010).

**Table 1.1** Summary of published phylogeographic work on large brown macroalgae, including major findings.

| Fucoids & kelps | Source | Key findings |
|---|---|---|
| *Ascophyllum nodosum* (N Atlantic) | (Olsen *et al.* 2010) | The species has survived the LGM on both sides of the Atlantic. The Brittany peninsula is a hotspot of genetic diversity worthy of conservation. |
| *Durvillaea antarctica* (Southern Ocean) | (Fraser *et al.* 2009a; Fraser *et al.* 2009b; Fraser *et al.* 2010) | Genetic homogeneity of subantarctic samples (10,000-km scales) is consistent with a post-glacial recolonization. The correspondence of genetic disjunctions with long beaches along the Chilean coast indicates that habitat discontinuity drives genetic isolation among established kelp populations. Apparently, rafting facilitates colonisation of unoccupied shores, but has limited potential to enhance gene-flow among established populations. New Zealand's "thonged" and "cape" morpho/ecotypes represent genetically distinct lineages and possibly reproductively isolated species. |
| *Durvillaea potatorum* (SE Australia) | (Fraser *et al.* 2009c) | The deep east–west divergence (attributed to a vicariant barrier during low Pleistocene sea levels) is consistent with morphological differences between 'western' and 'eastern' D. potatorum. Samples from western Tasmania and western Victoria are genetically monomorphic, suggesting postglacial expansion from a mainland refugium. |
| *Fucus distichus* (N Pacific & N Atlantic) | (Coyer *et al.* 2010) | At least two colonisations occurred from the older North Pacific populations to the North Atlantic between the opening of the Bering Strait and the onset of the Last Glacial Maximum. |
| *Fucus serratus* (N Atlantic) | (Hoarau *et al.* 2007) | Three disjunct refugia (SW Ireland, N Brittany-English Channel and NW Iberia) were recognized based on haplotype diversities and the presence of endemic haplotypes The later has not contributed to the post-glacial range expansion. |
| *Fucus spiralis /vesiculosus* complex (N Atlantic) | (Coyer *et al.* 2011) | Distinction of two spiralis entities. Contemporary populations of *F. spiralis* throughout the North Atlantic stem from a glacial refugium around Brittany involving F. spiralis High; F. spiralis South was probably unaffected by glacial episodes. The reticulate genealogy of the complex suggests historical introgression between species. |
| *Sargassum hemiphyllum* (NW Pacific) | (Cheang *et al.* 2010) | The allopatric form *chinense* is genetically distinct in all markers. Divergence is attributable to the vicariant event which resulted from the isolation of the Sea of Japan during the late Miocene. Possible geographical introgression of var. *chinense* plastids into S. *hemiphyllum*. |
| *Sargassum horneri* (NW Pacific) | (Hu *et al.* 2011) | Five distinct clades were recovered within S. horneri range. Clade I was restricted to Chinese marginal seas whereas clades II–V were discontinuously scattered around the main Islands of Japan. Two secondary contact regions were identified along the south Japan-Pacific coastline, which likely reflect historical glacial isolation and demographic expansion during the late Quaternary low sea levels. |
| *Lessonia nigrescens* (Chile) | (Tellier *et al.* 2009; Tellier *et al.* 2011) | Presence of two main divergent lineages with nearly disjunct distributions on each side of the 30ºS biogeographic transition zone. 12 populations belonging to the two lineages and sampled along a 50 km of coastline showed no hybridization or introgression, indicating complete reproductive isolation in natural conditions. These studies show that the two species are strictly segregated in space, raising interesting questions as to the mechanisms that limit sympatry at small spatial scales. |
| *Macrocystis pyrifera* (Chile & Southern Ocean) | (Macaya & Zuccarello 2010) | Only 5 related haplotypes were found among individuals collected along 4800 km of coastline, evidencing its shallow genealogy when compared with other macroalgal species. Some (not all) phylogeographic disjunctions correspond roughly to established biogeographic breaks. The low genetic diversity in northern Chile may be related to contemporary events (El Niño) while in southern Chile may reflect the effect of historical events (LGM). |

**The model species: *Fucus ceranoides* L.**

The horned wrack *Fucus ceranoides* L. (Fucales, Phaeophyceae) (Fig. 1.1a and 1.1b) is a perennial macroalga endemic to the Northeast Atlantic. This seaweed has a clear-cut distribution. It only occurs in estuarine environments, where steep salinity oscillations (from nearly fresh to nearly marine conditions) and alternating emersion/submersion periods occur throughout the tidal cycles (Fig. 1.1c). *F. ceranoides* is the dominant intertidal seaweed in these environments, being gradually replaced by its congeners *F. vesiculosus* and *F. spiralis* in the direction of the sea. The species frequently forms extensive (and often compact) belts along the margins of estuaries (Fig. 1.1d), where it grows attached to all sorts of hard substrata (walls, bridges, rocks, pebble, wood and even ropes).


**Figure 1.1** Morphology and habitat of *Fucus ceranoides* L. **(a)** Non-reproductive individual, showing the characteristic inflated leaves (Hardangerfjord, Norway). **(b)** Typical crown-shaped morphology of

receptacles (Bayonne, southern France). **(c)** Population in a small estuary (Brittany, France). **(d)** Dense, monospecific belt in a vertical wall (Bayonne).

*F. ceranoides* has a diplontic life-cycle, the haploid phase being reduced to the gametes (Fig. 1.2). Fertile individuals develop terminal, crown-shaped reproductive structures called receptacles (Fig. 1.1b), in which fertile cavities (conceptacles) are embedded. Most studies report *F. ceranoides* as dioecious (Lein 1984; Brawley 1992; Pérez-Ruzafa 2001; Billard *et al.* 2005a), although some authors from the early 20$^{th}$ century also report hermaphroditic individuals (Baker & Bohling 1916; Hamel 1931-1939). These were considered misidentifications or hybrids of *F. spiralis* and *F. ceranoides* by others (Powell 1963; Lein 1984), but more data may be necessary to determine the stability of the mating system of *F. ceranoides* throughout its range.

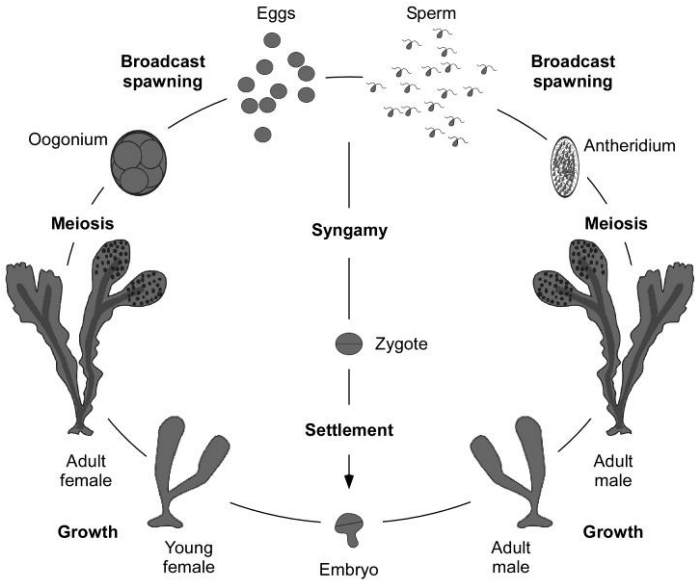


**Figure 1.2** Schematic representation of the life-cycle of *Fucus ceranoides* and other dioecious Fucoids.

Females conceptacles produce oogonia (each producing eight eggs), whereas male conceptacles contain antheridia (each releasing 64 antherozoids). Fertilization is external and follows gamete release (Pearson & Serrao 2006). As in other fucoids, broadcast spawning is synchronized by specific environmental cues, resulting in typically high fertilizing success of the eggs and zygote densities (Brawley 1992;

Pearson & Serrao 2006). Settlement competency is fast and attached zygotes develop directly into an adult alga.

*F. ceranoides* appears a very interesting model to investigate the interplay of intrinsic (habitat and life-history) and extrinsic (population subdivision and demographic history) factors shaping the population genetic structure of fucoid seaweeds (and marine restricted-dispersers in general). Its interest stems from several unusual characteristics. First, the species couples a strict dependency for a highly patchy habitat (estuaries) with the absence of dispersive (planktonic) developmental stages. Direct estimates of egg/zygote dispersal from the field confirm that fucoid propagules have much lower vagility when compared to invertebrate larvae (Serrão *et al.* 1997; Johnson & Brawley 1998; Dudgeon *et al.* 2001). Indeed, because of the negative buoyancy and the relatively short viability of eggs, and of the fast settlement competency of zygotes, these free-living stages disperse only locally, typically less than 5 mts from the spawning parent (Chapman 1995; Pearson & Serrao 2006). Because estuaries are normally separated by dozens of kilometres of open coast, this scale of dispersal is too small to allow inter-estuarine gene-flow. Similarly, the short longevity, rapid dilution and sensitivity to variations in salinity (Serrão *et al.* 1996a) should prevent migration of spawned sperm between populations.

Migration via rafting is also expected to be uncommon. Contrary to marine species, drifting thalli of *F. ceranoides* travel in a non-familiar, physiologically adverse environment, and the odds that they arrive to another suitable patch of estuarine habitat are presumably very low. Even then, drifters cannot *per se* enter or found a new population, since they lack the ability to reattach. Because the species is dioecious, at least one sexually reproducing individual of each sex needs to be in close contact to

produce *in situ* the zygotes that will eventually initiate a new population or result in effective gene-flow. Given these inherent dispersal restrictions, *F. ceranoides* is expected to display (as other large seaweeds) a strong phylogeographic and population genetic structure. However, because in this seaweed the scales of gametic dispersal (inside discrete estuaries, corresponding closely to the range of adult distribution and interaction) and migration via rafting (underlying inter-estuarine gene-flow and colonization) do not overlap, the roles of habitat isolation and rafting in structuring seaweed populations can be better appreciated.

Second, like many temperate organisms, *F. ceranoides* must have had a complex demographic history in the NE Atlantic. The extant distribution of *F. ceranoides* ranges from river Mondego in Portugal (41ºN) to Northern Norway [70ºN; (Lein 1984)] and Iceland (Munda 1999), being absent from the Baltic (Back *et al.* 1992) and most of the southern and eastern North Sea (Fig. 1.3). This vast distribution encompasses previously glaciated/emersed regions of Europe, and implies that the species has experienced important range adjustments in response to past climatic cycles. For instance, the colonization of Northern Europe is expected to have occurred only after the last glacial maximum (LGM), because presently the seaweed is not able to penetrate into the Arctic (Lein 1984). On the other extreme, the southern distributional limit of *F. ceranoides* coincides with the southern limit of an entire assemblage of cold-adapted fucoid and kelp species that typify the northern European shores, including *Ascophyllum nodosum*, *Pelvetia canaliculata*, *Fucus serratus*, *Himanthalia elongata*, *Halidrys siliquosa*, *Saccharina latissima* and *Laminaria hyperborea* (Luning 1990; Lima *et al.* 2007; Araújo *et al.* 2009).


**Figure 1.3** Modern geographic distribution of *Fucus ceranoides* (black shoreline, only inside estuaries). The approximate location of the shoreline (dotted line) and ice-sheets (pale grey) during the last glacial maximum (~20 ka BP) are also depicted.

Among other questions, analysis of *F. ceranoides* can contribute to better understand the genetic consequences of post-glacial range expansions in marine restricted-dispersers, and help clarify the evolutionary and conservation value of the marginal Iberian region for cold-temperate, canopy-forming macroalgae.

Third, the phylogenetic relationships among *F. ceranoides* and its close relatives remain unclear. ITS and mtDNA phylogenies of the genus *Fucus* could not resolve *F. ceranoides* from a species complex containing *F. vesiculosus, F. radicans, F. spiralis* and *F. virsoides*, suggesting a recent radiation or a reticulate history of gene exchange between the different species (Leclerc *et al.* 1998; Serrão *et al.* 1999; Coyer *et al.* 2006). Incomplete reproductive isolation and hybridization have been confirmed with nuclear markers for several *Fucus* pairs (Coyer *et al.* 2002a; Engel *et al.* 2005), and therefore *F. ceranoides* may potentially represent a good model to investigate the consequences of introgression for the genetic make-up of hybridizing marine species.

Finally, the genus *Fucus* has been receiving considerable attention of evolutionary biologists and molecular ecologists in the recent years (Leclerc *et al.* 1998; Serrão *et al.* 1999; Coyer *et al.* 2002b; Coyer *et al.* 2002a; Coyer *et al.* 2003; Billard *et al.* 2005a; Coleman & Brawley 2005; Engel *et al.* 2005; Coyer *et al.* 2006; Hoarau *et al.* 2007; Perrin *et al.* 2007; Tatarenkov *et al.* 2007; Coyer *et al.* 2008; Muhlin *et al.* 2008; Muhlin & Brawley 2009; Pearson *et al.* 2009; Pereyra *et al.* 2009; Coyer *et al.* 2010; Coyer *et al.* 2011; Moalic *et al.* 2011; Zardi *et al.* 2011). On one hand, many potentially useful molecular markers have already been developed. For instance, the complete mitochondrial (Secq *et al.* 2006) and chloroplast (Le Corguille *et al.* 2009) genomes have been sequenced, annotated and released, and many microsatellite loci have been developed for the congeners *F. vesiculosus* and *F. spiralis* (Engel *et al.* 2003; Wallace *et al.* 2004; Coyer *et al.* 2009). On the other hand, previous phylogenetic, phylogeographic and molecular ecological studies focusing on other species of *Fucus* provide an important body of work that should allow a broader perspective when interpreting the genetic patterns recovered in *F. ceranoides.*

# **CHAPTER II.** SURFING THE WAVE ON A BORROWED BOARD: RANGE EXPANSION AND SPREAD OF INTROGRESSED ORGANELLAR GENOMES IN THE SEAWEED *FUCUS CERANOIDES* L

# 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 (re)colonized 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

## 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 crossadaptive 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 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; Klopfstein *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 fucoid 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 (40ºN Lat., personal observation) to Northern Norway (70ºN 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. (Serrão *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 microsatellites (Billard *et al.* 2005a), 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 2.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 2.1). 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 2.1** *Fucus ceranoides* mtIGS haplotype frequencies and nucleotide diversity ($\pi$) for each geographic region and sampling site.

| ***Region*** *Population* | *ID* | *N* | *Clade* | *Shared haplotypes* | | | | *Private haplotypes* | *π (%)* |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A1 | C1 | I1 | I9 | | |
| **NW Iberia** | | **96** | **N** | | | | | | **0.891** |
| Viana do Castelo, Norte, **PT** | 1 | 24 | A | 21 | - | - | - | A2(2), A3 | 0.049 |
| Ria de Noia y Muros, W Galicia, **ES** | 2 | 24 | A | 17 | - | - | - | A4(7) | 0.087 |
| Ria de A Coruna, N Galicia **ES** | 3 | 24 | B | - | - | - | - | B1(15), B2(4), B3(3), B4(2) | 0.183 |
| River Porcia, W Asturias, **ES** | 4 | 24 | C | - | 20 | - | - | C2(2), C3, C4 | 0.083 |
| **Cantabrian Sea** | | **72** | **N** | | | | | | **0.212** |
| Ria de Villaviciosa, E Asturias **ES** | 5 | 24 | C | - | 17 | - | - | C5(6), C6 | 0.096 |
| Marismas de Santona, Cantabria, **ES** | 6 | 24 | C | - | - | - | - | C7(15), C8(9) | 0.099 |
| Bayonne, S Aquitaine, **FR** | 7 | 24 | C | - | 21 | - | - | C9(2), C10 | 0.049 |
| **Brittany & English Channel** | | **94** | **M** | | | | | | **2.168** |
| Anse de Saint Laurent, S Brittany, **FR** | 8 | 24 | C | - | 22 | - | - | C11, C12 | 0.067 |
| Penze, N Brittany, **FR** | 9 | 24 | I | - | - | 21 | - | I2(3) | 0.046 |
| Southampton, S England, **GB** | 10 | 22 | C | - | 22 | - | - | | - |
| Gweek, SW England, **GB** | 11 | 24 | I | - | - | 24 | - | | - |
| **Ireland & Wales** | | **95** | **I** | | | | | | **0.158** |
| Milford Haven, S Wales, **GB** | 12 | 24 | I | - | - | 6 | - | I3(18) | 0.079 |
| Caernarfon, N Wales, **GB** | 13 | 24 | I | - | - | 1 | 22 | I10 | 0.034 |
| Cork, Cork, **IE** | 14 | 23 | I | - | - | 21 | - | I4, I5 | 0.035 |
| Ramelton, Donegal, **IE** | 15 | 24 | I | - | - | 22 | - | I6, I7 | 0.034 |
| **Northern UK & Norway** | | **140** | **I** | | | | | | **0.125** |
| Oban, W Scotland, **GB** | 16 | 22 | I | - | - | 1 | 15 | I11(6) | 0.102 |
| Orkneys, N Scotland, **GB** | 17 | 22 | I | - | - | - | 22 | | - |
| Seaton Sluice, NE England, **GB** | 18 | 24 | I | - | - | 23 | - | I8 | 0.017 |
| Hardangerfjord, Hordaland, **NO** | 19 | 24 | I | - | - | 24 | - | | - |
| Trondheimsfjord, Nord-Trondelag, **NO** | 20 | 24 | I | - | - | 24 | - | | - |
| Folda, Nordland, **NO** | 21 | 24 | 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'-GTGCAAGAGCTGCGAAGTTT-3'; R 5'-CCCAAATGTAGGCGTATTGG-3') were

designed from the complete mitochondrial genome of *F. vesiculosus* (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'-CCAAGTTTTGAAAAGAAGCAA-3'; R 5'-TTGATAATGTTGTTGCGATTCA-3') were designed from the complete chloroplast genome of *F. vesiculosus* (Le Corguille *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 µL total volume containing 1× GoTaq Flexi buffer (Promega), 2.0 mM (mtIGS and cpORF501) or 1.0 mM (nPDI) $MgCl_2$, 125 µM each dNTP, 0.5 µM each primer, 1 U GoTaq® Flexi DNA Polymerase (Promega), and 2 µL of 1:100 diluted DNA template. An initial denaturation step (94 ºC, 5 min) was followed by 35 cycles of 94 ºC for 30 s, 58 ºC (mtIGS and cpORF501) or 61 ºC (nPDI) for 30 s and 72 ºC for 1 min and a final extension step (72 ºC, 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 $2 \times 10^6$ 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 ($\pi$) 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. 2.1). 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.

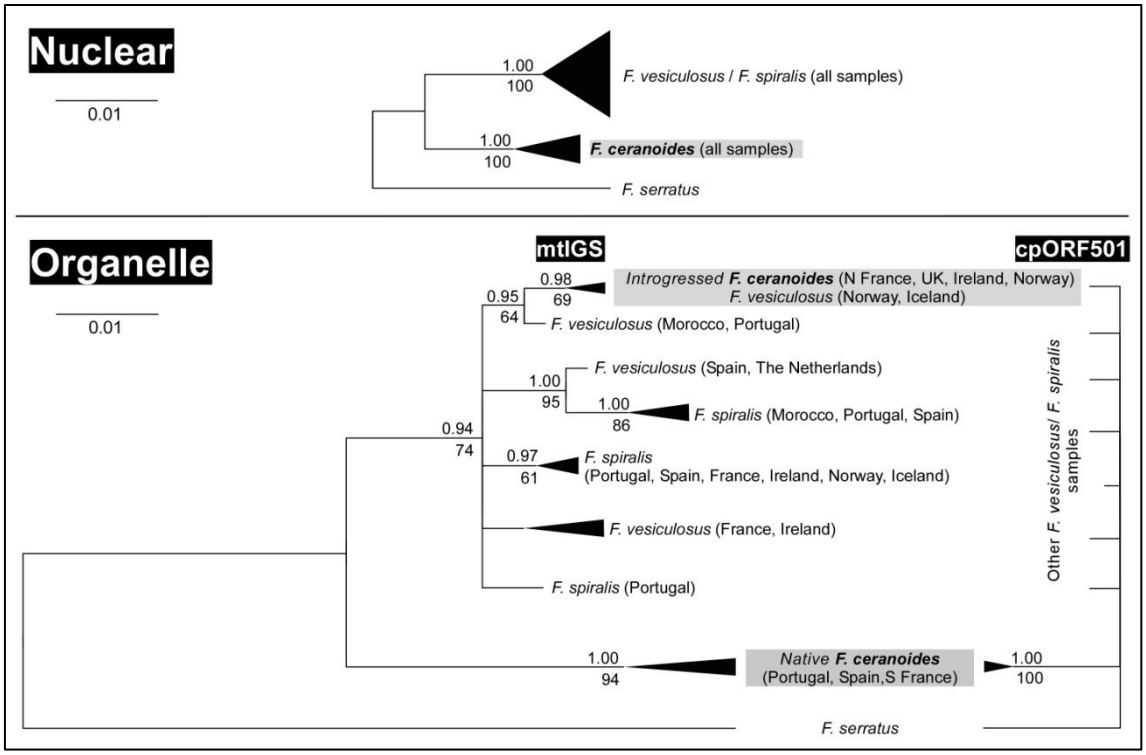


**Figure 2.1** Bayesian 50% majority-rule consensus trees based on the nPDI (Nuclear) and mtIGS & cpORF501 (Organelle) sequence data. Numbers above and below the branches are Bayesian posterior probabilities (>0.90) and maximum likelihood bootstrap support values (>60), respectively. All trees are built 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 organellar trees is highlighted in grey.

### *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 2.1 and Fig. 2.2). 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.

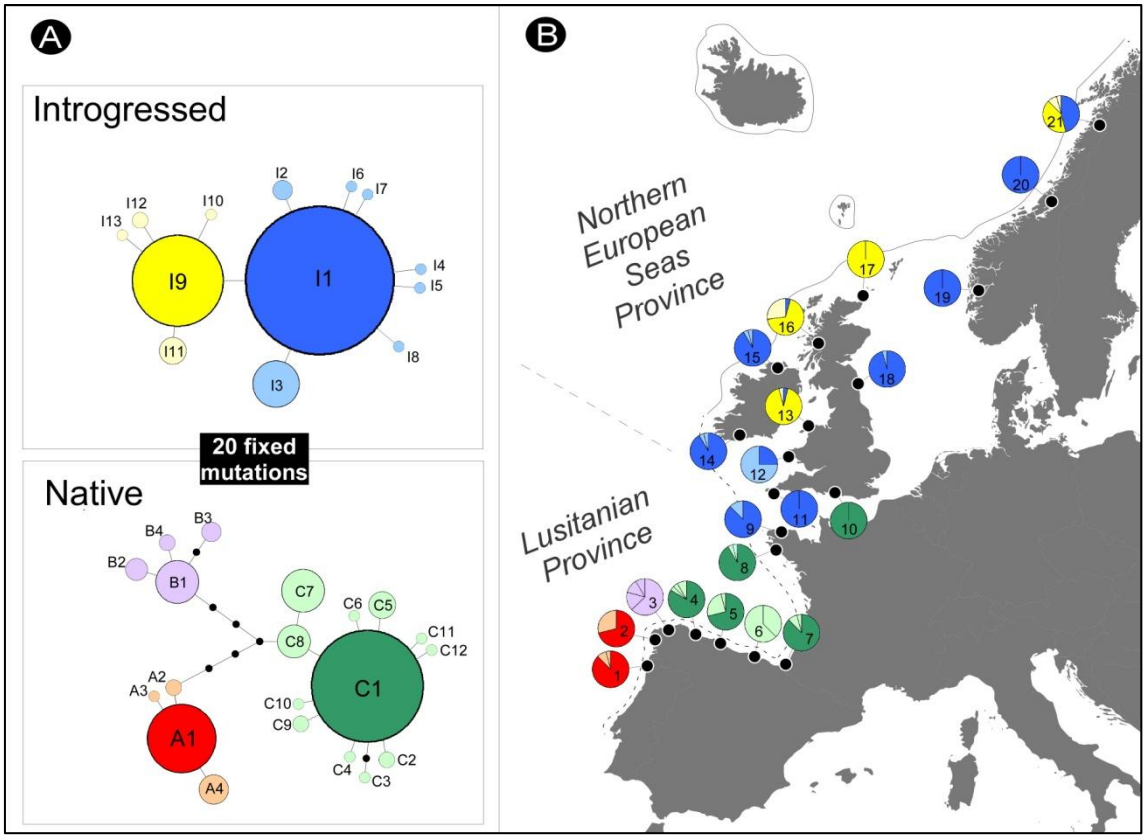


**Figure 2.2** *Fucus ceranoides* mtIGS haplotypes genealogy and distribution. **(a)** MtIGS haplotype parsimony networks. Sampled haplotypes are represented by circles sized to their frequency. Black dots represent inferred, unsampled haplotypes. Lineages are labelled by colour and letter [A, B, C and 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 2.1 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; Ménot *et al.* 2006)].

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. 2.2). 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 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 2.2) 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 ($F_{ST}$ = 0.987).

**Table 2.2** 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 | |

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 ($F$ > 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* organellar 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 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.* 2005a) 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 y 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 ºC 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 mtIGS 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 palaeoclimatic 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 (Ménot *et al.* 2006). Such post-glacial, 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 3º of the c. 30º 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 $F_{SC}$ 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. 2.1; (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 palaeo-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 ¼ 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 cytoplasmatic 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; Kuriiwa *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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## Supplementary Material

**Table 2.S1** Geographic origin of *Fucus* spp. samples (N= 35) sequenced for the nPDI, mtIGS and cpORF501 loci.

| ***Species*** |
| --- |
| *Site, Administrative region,* ***Country*** |
| ***F. serratus*** |
| Constantine Bay, England, **United Kingdom** |
| ***F. ceranoides*** |
| Viana do Castelo, Norte, **Portugal** |
| Ria de Noia y Muros, Galicia, **Spain** |
| River Porcia, Asturias, **Spain** |
| Marismas de Santoña, Cantabria, **Spain** |
| Bayonne, Aquitaine, **France** |
| Penze, Brittany, **France** |
| Gweek, England, **United Kingdom** |
| Caernarfon, Wales, **United Kingdom** |
| Ramelton, Donegal, **Ireland** |
| Folda, Nordland, **Norway** |
| ***F. vesiculosus*** |
| Oualidia, Doukkala-Abda, **Morocco** |
| Tavira, Algarve, **Portugal** |
| Alcochete, Lisboa, **Portugal** |
| Viana do Castelo, Norte, **Portugal** |
| Ortigueira, Galicia, **Spain** |
| Marismas de Santoña, Cantabria, **Spain** |
| Santec, Brittany, **France** |
| Cape Gris-Nez, Nord-Pas-de-Calais, **France** |
| Hoek van Holland, South Holland, **The Netherlands** |
| Portaferry, Northern Ireland, **United Kingdom** |
| Langesund, Telemark, **Norway** |
| Reykjavik, Capital Region, **Iceland** |
| ***F. spiralis*** |
| Oualidia, Doukkala-Abda, **Morocco** |
| Albufeira, Algarve, **Portugal** |
| Cabo Raso, Lisboa, **Portugal** |
| Viana do Castelo, Norte, **Portugal** (high shore morphotype) |
| (low shore morphotype) |
| Castello beach, Asturias, **Spain** |
| Marismas de Santoña, Cantabria, **Spain** |
| Santec, Brittany, **France** |
| Cape Gris-Nez, Nord-Pas-de-Calais, **France** |
| Portaferry, Northern Ireland, **United Kingdom** |
| Langesund, Telemark, **Norway** |
| Reykjavik, Capital Region, **Iceland** |

**CHAPTER III.** DRIFTING FRONDS AND DRIFTING ALLELES: RANGE DYNAMICS, LOCAL DISPERSAL AND HABITAT ISOLATION SHAPE THE POPULATION STRUCTURE OF THE ESTUARINE SEAWEED *FUCUS CERANOIDES* L.

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

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**Abstract**

**Aim** The seaweed *Fucus ceranoides* is restricted to spatially discrete estuarine habitats, lacks planktonic dispersal phases, and is therefore expected to exhibit strong population differentiation. Its cold-temperate affinities and mtDNA variation imply that the Northern part of the species range, where *F. ceranoides* is now ubiquitous, was recently colonized after the onset of the last deglaciation, potentially resulting in areas with greater genetic homogeneity. Here we examine the population structure of *F. ceranoides* to test these predictions, emphasizing the contrasting genetic signatures of limited dispersal in refugial versus recently colonized regions. **Location** North-eastern Atlantic estuaries from Portugal to Norway. **Methods** 504 individuals from 21 estuarine sites spanning the entire range of *F. ceranoides* were sampled and genotyped for 9 microsatellite loci. Population structure was inferred from several genotypic and allele-frequency analyses. Geographical patterns of genetic diversity were used to reconstruct

the historical biogeography of the species. **Results** Genetic diversity and regional population differentiation showed a consistent decline with increasing latitude. Southernmost populations harboured most of the endemic variation, whereas the Northern populations (>55ºN) were almost fixed for the same alleles across loci. In Southern and Central regions of its distribution, *F. ceranoides* showed striking population subdivision, with many of the sampled estuaries corresponding to coherent genetic units that were easily discriminated from one another with standard clustering methods. **Main conclusions** The geographical pattern of genetic diversity supports the long-term refugial status of Iberia and a post-glacial range expansion of *F. ceranoides* into previously glaciated latitudes. Despite this capacity to colonize newly available habitats, the genetic structure of *F. ceranoides* outside the recently (re)colonized range reveals that gene flow between populations is extremely low. This study provides a remarkable example of how infrequent and spatially limited dispersal can have contrasting effects at the scales of meta-population (connectivity) *versus* range dynamics (habitat tracking), and how dispersal restrictions can result in either genetic divergence or homogeneity depending on the maturity and demographic conditions of the populations.

**Keywords** estuary, *Fucus ceranoides*, microsatellites, Pleistocene refugium, population structure, post-glacial expansion, rafting

## Introduction

Over the Quaternary period, the historical distribution of ecosystems and species has been largely mediated by the alternation of cold glacial and temperate interglacial conditions (Hofreiter & Stewart 2009). During glacial peaks, such as the last glacial

maximum (LGM, ~20000 y BP), temperate taxa typically persisted in southern refugial areas, whereas interglacials (such as the present one) allowed the (re)colonization of more northern latitudes (Taberlet *et al.* 1998; Hewitt 1999; Petit *et al.* 2002). This classical glacial contraction/interglacial expansion model is central to phylogeographical research, and helps explain the common poleward decrease of genetic diversity exhibited by terrestrial taxa (Hewitt 1999; Hampe & Petit 2005). Species responses to climatic oscillations have nevertheless taken more diverse and idiosyncratic forms (Stewart & Lister 2001; Gómez & Lunt 2007; Svenning *et al.* 2008), depending on the specific ecological adaptations and life-history traits displayed by individual taxa (Bennett & Provan 2008; Bhagwat & Willis 2008; Stewart & Dalen 2008; Stewart *et al.* 2010).

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

Strict analogies between terrestrial and marine environments may be inappropriate, because biological assemblages, geographical templates, environmental gradients and dispersal patterns are fundamentally different between the two realms (Graham *et al.* 2003; Kinlan & Gaines 2003; Lomolino *et al.* 2005; Harley *et al.* 2006).

The inferred location of marine refugia, for instance, frequently differs from terrestrial settings. The ice-free palaeo-shorelines of Brittany, the English Channel and SW Ireland have consistently been recognized as northern, peri-glacial refugia (in addition to more southern refugia) for a range of invertebrate and seaweed species (Provan *et al.* 2005; Gómez *et al.* 2007a; Hoarau *et al.* 2007; Remerie *et al.* 2009; Olsen *et al.* 2010), despite the absence of most of their modern terrestrial counterparts. Also, instead of showing signatures of stable rear edges, southernmost populations of some coastal species are genetically impoverished [but see (Provan & Maggs 2011)], presumably as a consequence of the progressive marginality of the habitat and continuing demographic instability brought about by the inter-glacial warming (Coyer *et al.* 2003; Olsen *et al.* 2010).

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

The cold-temperate, European endemic fucoid seaweed *Fucus ceranoides* L. (horned wrack) is perennial and dioecious. It is restricted to estuarine intertidal areas under fluctuating salinities, where it forms relatively small and isolated populations.

Since fucoids have no planktonic dispersal stages and gamete dispersal is very local [<50 m; (Chapman 1995; Serrão *et al.* 1997; Dudgeon *et al.* 2001)], its populations presumably persist mostly via self-recruitment. The modern distribution of *F. ceranoides* ranges from the River Mondego in Portugal (41ºN) to northern Norway (70ºN) and Iceland (Lein 1984; Munda 1999), encompassing both previously glaciated and non-glaciated regions of Europe.

A recent mtDNA survey showed that the southernmost *F. ceranoides* populations of NW Iberia harbour two endemic, highly differentiated lineages, indicating a possible Pleistocene climatic refugium for the species [(Neiva *et al.* 2010); reproduced in Fig. 3.1). Outside Iberia, however, over large areas *F. ceranoides* exhibits an introgressed, *F. vesiculosus*-derived mtDNA lineage associated with the species post-glacial, poleward range expansion (Neiva *et al.* 2010). The extent of introgression and low polymorphism limited the phylogeographical resolution of the mtDNA marker. Because sequence and typing data show that organellar capture was not accompanied by nuclear introgression (Billard *et al.* 2005a; Neiva *et al.* 2010), here we examine, using nuclear microsatellite markers, the population genetic structure of *F. ceranoides* throughout its whole distributional range. Our aim is to reconstruct the historical biogeography of *F. ceranoides*, and to evaluate the effects of limited dispersal and recent range shifts on the geographical organization of its genetic variation. We were particularly interested in testing the prediction that *F. ceranoides* exhibits considerably more diversity and among-population differentiation in Southern (refugial) than in Northern (recently colonized) regions.

## Material and Methods

*Sampling, DNA extraction and genotyping*

*F. ceranoides* populations were sampled during 2008 from 21 estuaries from Portugal to Norway, covering the entire current distribution of the species in the northeast Atlantic (Fig. 3.1 and Table 3.1) with the exception of Iceland, where different *Fucus* species were found at the sites where *F. ceranoides* was expected. Minimum marine distances between adjacent populations ranged from 140 to ≈ 1200 km. At each site, 5-10 cm tips of apical vegetative tissue were excised from 22-24 individuals sampled along a 50-200 m linear or random walk. The protocols for DNA extraction are detailed in Neiva *et al*. (2010).

After testing in a small panel of *F. ceranoides* the cross-amplification, scoring and polymorphism of 25 microsatellite loci previously developed for other *Fucus* species (Engel *et al.* 2003; Wallace *et al.* 2004; Perrin *et al.* 2007; Coyer *et al.* 2009), 9 microsatellite loci were selected and amplified to generate multi-locus genotypes for all *F. ceranoides* individuals (see Table 3.S1 for sources, primers and amplification details). Polymerase chain reactions (PCRs) were performed in 10 μL total volume containing 1× GoTaq Flexi buffer (Promega), 1.5-2.0 mM $MgCl_2$, 125 μM each dNTP, 0.2 μM of labelled (FAM, NED or HEX) forward primers, 0.5 μM of reverse primers, 1U GoTaq® Flexi DNA Polymerase (Promega), and 1 μL of 1:100 diluted DNA template. In all PCRs an initial denaturation step (94 ºC, 5 min) was followed by *n* cycles of 94 ºC for 30 s, a primer-specific annealing temperature ($T_a$) for 15 s and 72 ºC for 30 s, ending with a final extension step at 72 ºC for 10 min (Table 3.S1). Amplified fragments were separated in an ABI PRISM 3130xl (Applied Biosystems, CCMAR Portugal) automated capillary sequencer. Alleles were manually scored in STRand (Toonen & Hughes 2001) using the 350 ROX™ size standard (Applied Biosystems).

**Table 3.1** Genetic diversity and $F_{IS}$ estimates in *Fucus ceranoides* at each sampling site and within selected geographical regions (in bold), based on nine microsatellite loci. N, number of individuals genotyped in each population; A, mean number of alleles per locus; $H_E$, Nei's gene diversity; $H_o$, observed heterozygosity; $F_{IS}$, multi-locus inbreeding coefficient. The right column shows previously published data on the mtIGS locus (Neiva *et al.* 2010). Lineages and haplotypes are listed for each locality and region and coded as in Figure 3.1. Absolute frequencies of haplotypes are in parenthesis (if N>1).

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

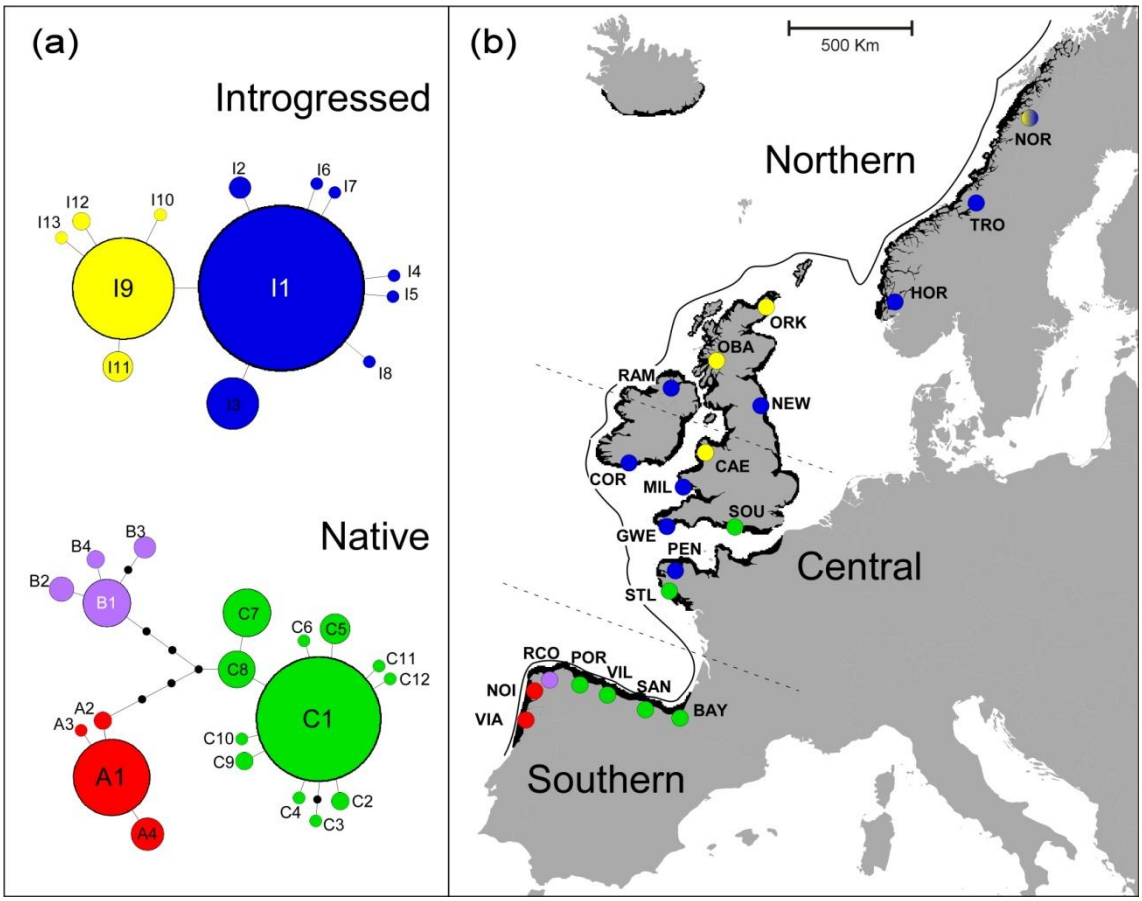


**Figure 3.1** Distributional range of *Fucus ceranoides*, sampling sites and genetic subdivision inferred from a mitochondrial intergenic spacer, mtIGS (Neiva *et al.* 2010). **(a)** Parsimony networks of native (lineages $N_A$- red, $N_B$- purple, $N_C$- green) and *F. vesiculosus*-derived (introgressed, lineage I- blue and yellow) mtIGS haplotypes. Sampled haplotypes are represented by circles sized to their frequencies; black dots represent inferred, unsampled haplotypes. Links represent a single nucleotide change. **(b)** Sampling locations of *F. ceranoides* and distribution of mtIGS lineages. The black contour depicts the distribution of *F. ceranoides* (only inside estuaries along those coastlines), the straight dashed lines delimit the geographical subdivisions considered (Southern, Central and Northern regions), and the circles represent the sampling localities coded as in Table 3.1 and coloured according to the mtIGS lineages present. The solid line represents the approximate LGM shoreline. Adapted from Neiva *et al*. (2010).

*Data analysis*

Summary statistics of genetic diversity within populations, including allele frequencies, mean allelic richness ($A$), Nei's gene diversity ($H_E$), observed heterozygosity ($H_O$) and inbreeding coefficients ($F_{IS}$), were calculated with GENETIX 4.05 (Belkhir *et al.*, 1996-2004). The same statistics were also computed for three selected regions, each encompassing a set of seven populations, broadly corresponding to the Southern

(Iberia), Central (Brittany, English Channel, Celtic and Irish Seas) and Northern (>55ºN) parts of the *F. ceranoides* range.

Genetic structure was assessed using both population (allele frequency-based) and individual (genotype-based) approaches. Pairwise $F_{ST}$ [$\theta$; (Weir & Cockerham 1984)] was estimated with GENETIX 4.05 (Belkhir *et al.* 1996-2004) and D [$D_{est}$; (Jost 2008)] was estimated with SMOGD 1.25 (Crawford 2010). Because $H_E$ was very variable among populations (see RESULTS), isolation by distance (IBD) was analysed with $D_{est}$ instead of $F_{ST}$-based measures. IBD was evaluated for full and regional data-sets using reduced major axis regressions of pairwise estimates of D against minimum marine distances, as measured in Google Earth 5.1. The statistical significance of the genetic/geographical associations (1000 randomizations, $P$ = 0.05) was assessed with Mantel tests in IBDWS (Jensen *et al.* 2005).

In order to illustrate the degree of association between genotypic variation and geography, a neighbour-joining (NJ) network was generated from a matrix of pairwise genetic distances (Cavalli-Sforza & Edwards 1967) of individuals (genotypes), using POPULATIONS 1.2 (Langella 1999). Population genetic structure was further tested at the regional level (Southern and Central regions only) with a Bayesian, model-based genetic admixture analysis implemented by STRUCTURE 2.3 (Pritchard *et al.* 2000; Falush *et al.* 2003). Individuals were combined into one dataset for analysis, without any *a priori* population assignments and admixture was allowed. Each number of assumed populations ($K$, set sequentially from 1 to 9) was run five times using a burn-in of 100000 iterations and a run-length of 500000 iterations, which was determined to be sufficient to have consistent results. The "true" value of $K$ was inferred both from the posterior probability of the data, hereafter referred to as $L(K)$, and following the $\Delta K$

choice criterion (Evanno *et al.* 2005), better suited to detect heterogeneous patterns of dispersal or co-ancestry.

Recent migration rates among Southern and Central populations were estimated using a Bayesian MCMC analysis with BAYESASS 1.3 (Wilson & Rannala 2003). Individuals were pre-assigned to populations based on sampling location. The analysis parameters were a burn-in of 1000000 iterations, a run-length of 3000000 iterations (sampling every 2000), and the default delta value of 0.15 for allele frequency, migration rate and level of inbreeding.

**Results**

A total of 500 multi-locus genotypes of *Fucus ceranoides* from the 21 estuarine sites along the NE Atlantic were produced using the 9 selected microsatellite loci. Microsatellite polymorphism varied from 2 to 15 alleles per locus, with a mean (total allelic richness) of 8.56, dropping to 5.33 if only the alleles with a global frequency above 0.01 were considered. Most populations were monomorphic (or nearly fixed) for at least one locus, frequently more, especially outside Iberia (Fig. 3.S1). $H_E$ ranged from 0 (the northernmost population, NOR, fixed for a single allele at all 9 loci analysed) to 0.463 (BAY) (Table 3.1). $H_O$ was not significantly different from $H_E$ with the exception of the GWE population, where heterozygote excess was detected at locus L58.

Both $A$ and $H_E$ estimators of genetic diversity showed a marked decline with latitude, at the population level but especially at the regional level (Fig. 3.S2). On average, $H_E$ was ~ 6 times higher in the Southern populations ($H_E$= 0.357 ± 0.100) as compared with the Northern populations ($H_E$= 0.060 ± 0.055), and ~ 7 times higher when compared for the regions as a whole (Fig. 3.2a). The Central populations showed

intermediate levels of genetic diversity but larger variation among sites ($H_E$= 0.161 ± 0.120).

All but three pairs of populations showed significant differentiation ($P$<0.05), with strikingly extreme values of $F_{ST}$ ranging from 0.007 (the two northernmost populations, essentially fixed for the same genotype) to 0.954 (SOU vs. NOR, nearly fixed for different genotypes) (Table 3.S2). Globally, 184 (87.6%) of the 210 pairwise $F_{ST}$ estimates were greater than 0.3, 131 (62.4%) greater than 0.5, and 51 (24.3%) greater than 0.7. Absolute population differentiation, as measured by $D_{est}$, ranged from ≤0.001 (several pair-wise comparisons among Northern populations), to 0.582 (NOI vs. STL; Table 3.S2). Within regions, the highest $D_{est}$ values were found in population pairs from the Southern region, but this was also the region with more variable $D_{est}$ (0.070-0.556; Fig. 3.2b). Contrastingly, $D_{est}$ values were lower and more homogeneous within Central Europe (0.024-0.180), and basically null (<0.029) among Northern populations.

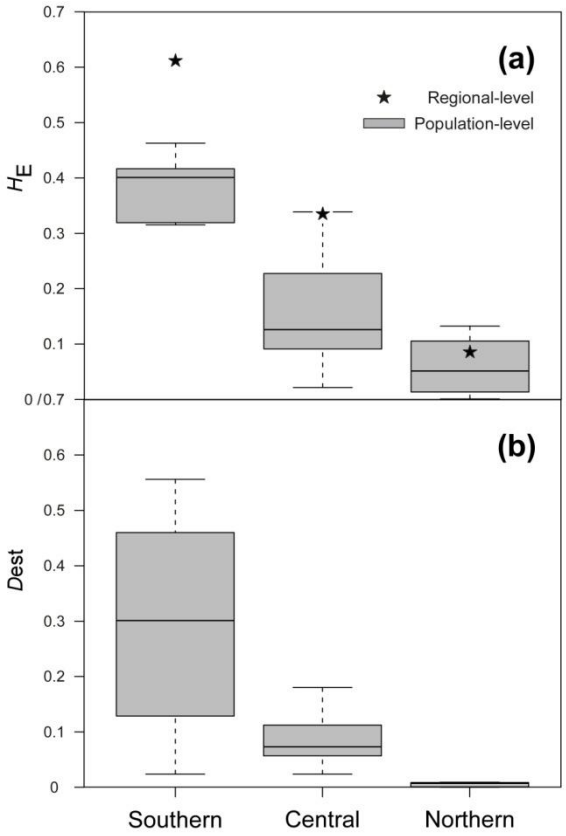


**Figure 3.2** Genetic diversity and differentiation of populations of *Fucus ceranoides* within Southern, Central and Northern regions. **(a)** Nei's gene diversity ($H_E$) at population (box-plot, N=7) and regional (stars) levels. **(b)** Box-plot of pairwise differentiation of populations ($D_{est}$) within regions (N=70). Box-plots depict the median (horizontal line), the 25$^{th}$ and 75$^{th}$ percentiles (bottom and top of the box) and the minimum/maximum values (vertical dashed lines).

No pattern of IBD was detected for the whole dataset ($P$=0.090), although a weak significant relationship ($P$=0.002) was observed for the sub-set of "Core" populations, after the 6 most peripheral populations were removed from the analysis, including the highly differentiated NW Iberian populations of VIA, NOI and RCO, as well as the nearly identical, geographically distant Norwegian populations (Fig. 3.3a). At the regional level, a nearly significant IBD relationship was observed within Iberia ($P$=0.053), but not in the English Channel ($P$=0.309; Fig. 3.3b). This analysis was not relevant for the Northern region given the very low level of differentiation between populations.

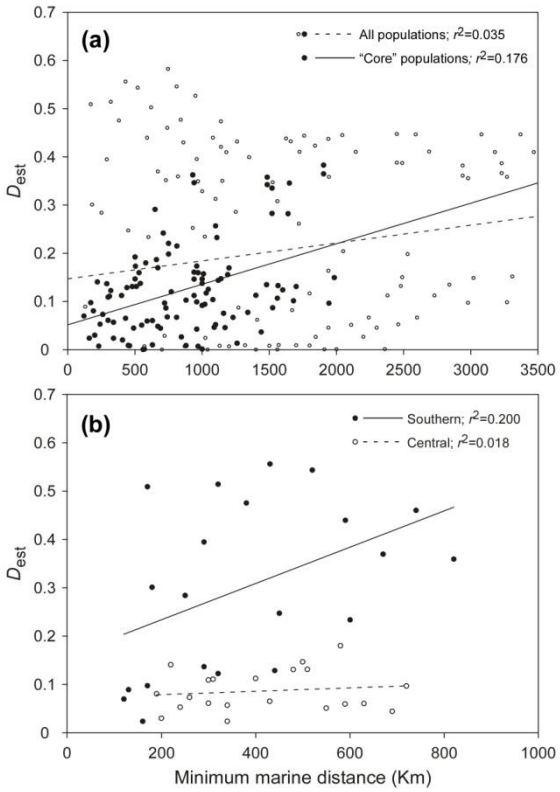


**Figure 3.3** Isolation by distance in *Fucus ceranoides.* Estimates of pairwise differentiation ($D_{est}$) plotted against minimum marine distance in kilometres for **(a)** all populations (dashed line) and "core" populations (solid line), and **(b)** populations from Southern (dashed line) and Ccentral (solid line) regions of the species' range. The regressions are: $y = 3.704 \times 10^{-5}x + 0.147$ (all populations), $y = 8.410 \times 10^{-5}x + 0.051$ ("core" populations), $y = 3.752 \times 10^{-4}x + 0.159$ (Southern region) and $y = 3.454 \times 10^{-5}x + 0.072$ (Central region).

The NJ network (Fig. 3.4) highlights the greater differentiation of several Iberian genotypic/ geographical/ mitochondrial clusters (e.g. VIA/NOI, RCO, POR/VIL) and higher regional diversity within Iberia (Southern region) compared with the Central/Northern cluster.

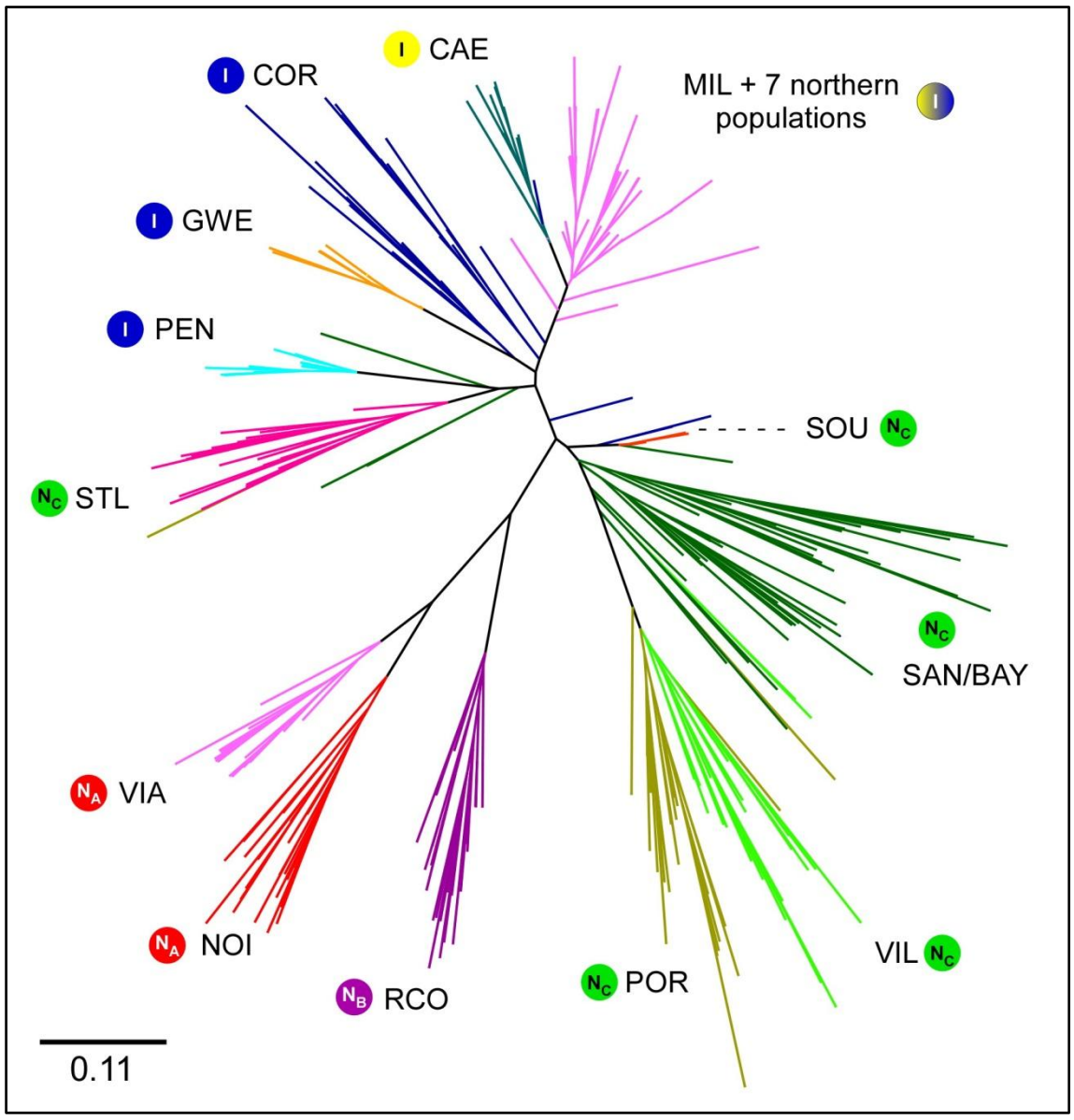


**Figure 3.4** Neighbour-joining network of genotypes of *Fucus ceranoides* using Cavalli-Sforza & Edwards's (1967) pairwise distances. The inferred genotypic/ geographical clusters are shown in different colours and are accompanied by a circle representing the mtIGS clades present. Population codes and mtIGS clade designations are given in Table 3.1.

The network also shows that *F. ceranoides* multi-locus genotypes were strongly clustered geographically. Throughout the Southern and Central parts of the distributional range there was a remarkable correspondence between genotype clusters and single (or occasionally two) estuarine sites.In the Northern region the pattern was completely opposite, with most individuals basically fixed across loci for the same alleles thus forming a single cluster with no geographical resolution. All genotypes from Brittany northwards (i.e. all Central and Northern regions, excluding Sou) formed a rather cohesive cluster that was well differentiated from the Southern genotypes. Within the latter, the southernmost Iberian genotypes (VIA/NOI and RCO) formed the clusters most differentiated from all others in the species.

Within Southern and Central regions, the STRUCTURE clustering analysis of the multilocus genotypes also revealed striking population subdivision down to the estuarine level (Fig. 3.5). *F. ceranoides* populations were hierarchically structured within Iberia (Fig. 3.5a). Based on the ΔK criterion (Evanno *et al.* 2005) the highest level of genetic/geographical sub-division occured between VIA/NOI and the remaining populations of Iberia (K=2), with VIA/NOI, RCO, POR/VIL, and SAN/BAY (K=4) representing a weaker level of population subdivision (Fig. 3.S3a). Alternatively, the L(K) criterion (Pritchard *et al.* 2000) resolved 6 distinct genetic clusters matching specific estuarine sites (with the exception of VIA and NOI that clustered together), although suggesting an appreciable degree of admixture between SAN and BAY sites. In the Central region, all the 7 estuarine sites sampled were largely resolved as distinct genetic clusters irrespective of the choice criterion used (Fig. 3.S3b).

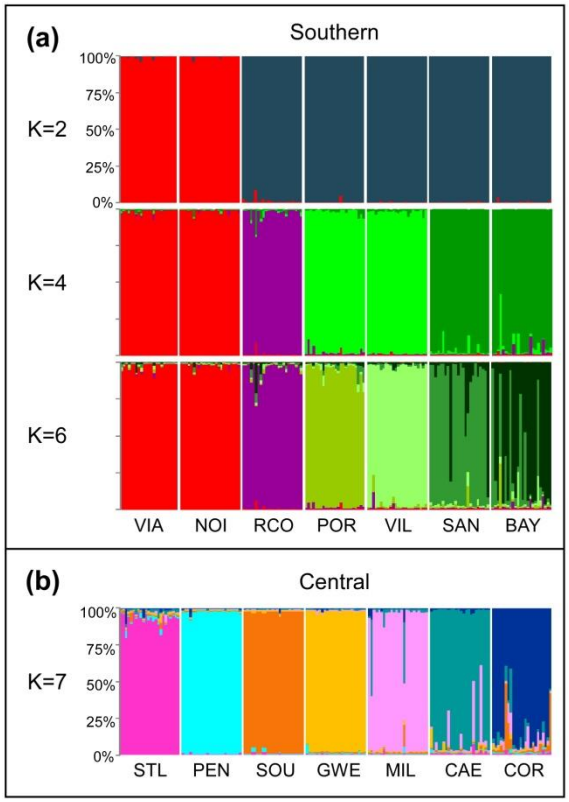


**Figure 3.5** Genetic subdivision of *Fucus ceranoides* in **(a)** Southern, and **(b)** Central regions based on STRUCTURE. Shown are the proportions of individual multilocus genotypes assigned to each of the K virtual clusters, as illustrated by the different colours. Population codes are given in Table 3.1.

The Bayesian inference of migration rates indicates that, among populations within Southern or Central regions, recent immigration was generally lower than 0.02 individuals per generation (Table 3.S3), i.e., that these populations are evolving rather independently. Nevertheless, estimated migration rates from SAN to BAY (Southern region) and from MIL to CAE (Central region), contiguous in both cases, were not negligible (m= 0.292 ind. gen$^{-1}$). These two pairs of populations do not, however, share the same mtIGS haplotypes (Table 3.1), showing lack of connectivity, at least for females, between these neighbouring populations.

## Discussion

*Historical range-dynamics*

A clear poleward decrease in allelic richness, gene diversity and within-region population differentiation was revealed by the comparison of Southern, Central and

Northern populations of *Fucus ceranoides*. The Southern region (i.e. Iberia) harbours many private alleles (in addition to two endemic mtIGS lineages) and populations there were grouped hierarchically in small shoreline sectors (<200 km). These were strongly differentiated from each other and from more northern samples. This pattern of "southern richness" is characteristic of temperate terrestrial taxa that have persisted throughout past glacial conditions in southern refugia (Hampe & Petit 2005), and therefore supports the hypothesis of long-term persistence of *F. ceranoides* in NW Iberia, possibly N Iberia as a whole.

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

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

The expected post-glacial range expansion of *F. ceranoides* into previously glaciated latitudes is strongly supported, as the extremely low microsatellite gene diversity and lack of population differentiation in the Northern region represents good empirical evidence of a severe and recent colonization sweep (Pascual *et al.* 2001; Excoffier *et al.* 2009). Indeed, Northern populations were extremely homogeneous despite the distance between many samples exceeding 1000 km. Although the microsatellite data support an expansion originating from the Central region, they fail to clearly delineate colonization pathways. Still, the distribution of the two most common haplotypes (I1 and I9, see Neiva *et al.* 2010) suggests that colonization proceeded in two different phases and routes, as in the estuarine mysid *Neomysis integer* (Remerie *et*

*al.* 2009). The first wave migrated northwards following the submersion of the Celtic and Irish Seas, during which the originally introgressed haplotype I1 was replaced by the derived haplotype I9, which eventually spread further north along Scotland. After the re-establishment of the passage between the Channel and the North Sea (around 7.5 ky BP) a second wave also bearing the "ancestral" haplotype I1 expanded along the previously emerged English Channel, then northwards along the east coast of England and ultimately reached the Norwegian fjords.

The "southern richness-northern purity" genetic pattern of *F. ceranoides* contrasts with those found in other cold-temperate fucoids with which it shares the same general distribution in the NE Atlantic. The highest levels of genetic diversity in *F. serratus* and *Ascophyllum nodosum* are found in Brittany, the English Channel and southwestern Ireland, i.e., in more central regions of their extant distributions, while populations at their common, southern distributional boundary are comparatively impoverished (Coyer *et al.* 2003; Hoarau *et al.* 2007; Olsen *et al.* 2010). These species have more restricted horizontal and vertical distributions in Iberia (Pearson *et al.* 2009; Araújo *et al.* 2011), and also exhibit more unstable demographies and range dynamics there (Arrontes 1993; Arrontes 2002; Fernández & Anadón 2008; Viejo *et al.* 2010). The highest levels of genetic diversity and endemism for *F. ceranoides* observed in this same southernmost region show that the area still represents a stable rear edge that has not turned into a marginal habitat during the course of the present interglacial. Unique genetic variation was also detected in Iberian populations of the red seaweed *Chondrus crispus* (Provan & Maggs 2011), suggesting that this restricted and marginal region may represent an important repository of genetic diversity in several other intertidal organisms with similar geographic distributions.

*Drifting fronds and drifting alleles*

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

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

Gamete dispersal is very local in fucoid algae and thus *F. ceranoides* individuals will mostly interact and reproduce within the discrete, isolated patches of estuarine habitat they inhabit. Within populations, no significant departures from Hardy-

Weinberg equilibrium were found, suggesting random gamete mixing at the scale of the sampling design (i.e. for non-neighbouring individuals along 50-200 m linear distances). Given the larger dispersal capacity of *Fucus* sperm (>10 m) than eggs [most settle within 0.5 m, (Serrão *et al.* 1997), such local mixing within *F. ceranoides* populations is probably mediated by sperm dispersal rather than eggs. Yet once released, even in the odd case of sperm transport beyond the estuary, it is doubtful that they could mediate inter-estuarine gene flow given their short longevity, rapid dilution and sensitivity to variations in salinity (Serrão *et al.* 1996a).

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

Inter-estuarine gene flow requires contact between a single immigrant and an established population. The regional population genetic structure of *F. ceranoides* is characterized by fixed haplotypic/allelic differences between populations and lack of IBD, which suggests that historical and recurrent (drift) processes contributing to population differentiation are weakly counteracted by on-going gene flow. Effective migration via rafting must thus be rare and relatively erratic over most spatial scales (Thiel & Haye 2006). Likely, the effects of isolation are reinforced by drift under small

effective population sizes (Turner *et al.* 2002; Coyer *et al.* 2008). These are expected from the typically small size of estuarine patches, habitat instability over ecologically and geologically scales (Attrill & Rundle 2002) and large variance in the reproductive success of individuals (Vernet & Harper 1980; Billard *et al.* 2005b; Pearson & Serrao 2006).

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

*Conclusions*

The palaeoclimatic oscillations in the N Atlantic have clearly driven important biogeographical shifts in *F. ceranoides* and played a significant role in shaping its genetic make-up. Our study supports the status of Iberia as a long-term (multiple

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

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

**Acknowledgements**

The authors thank all the people who contributed with samples: Ignacio Barbara, Claire Daguin, Emmanuelle Billard, Christophe Destombe, John Davenport, Christine Maggs, Phil Budd, Richard Joseph, Paul Brazier, Martin Wilkinson, Holly Brown, Malcolm Thomson, Vivian Husa, Herre Stegenga, Sara Marsham and Karl Gunnarson. We thank Marta Valente, Xana Ramos and Céline Madeira for their genotyping work and Alice Neiva, Duarte Neiva and Joana Costa for field assistance. This study was supported by research projects of the Portuguese Fundação para a Ciência e Tecnologia (FCT), and by a PhD grant SFRH/BD/31017/2006 from FCT co-funded by FSE to J.N.

## Supplementary material

## Figures

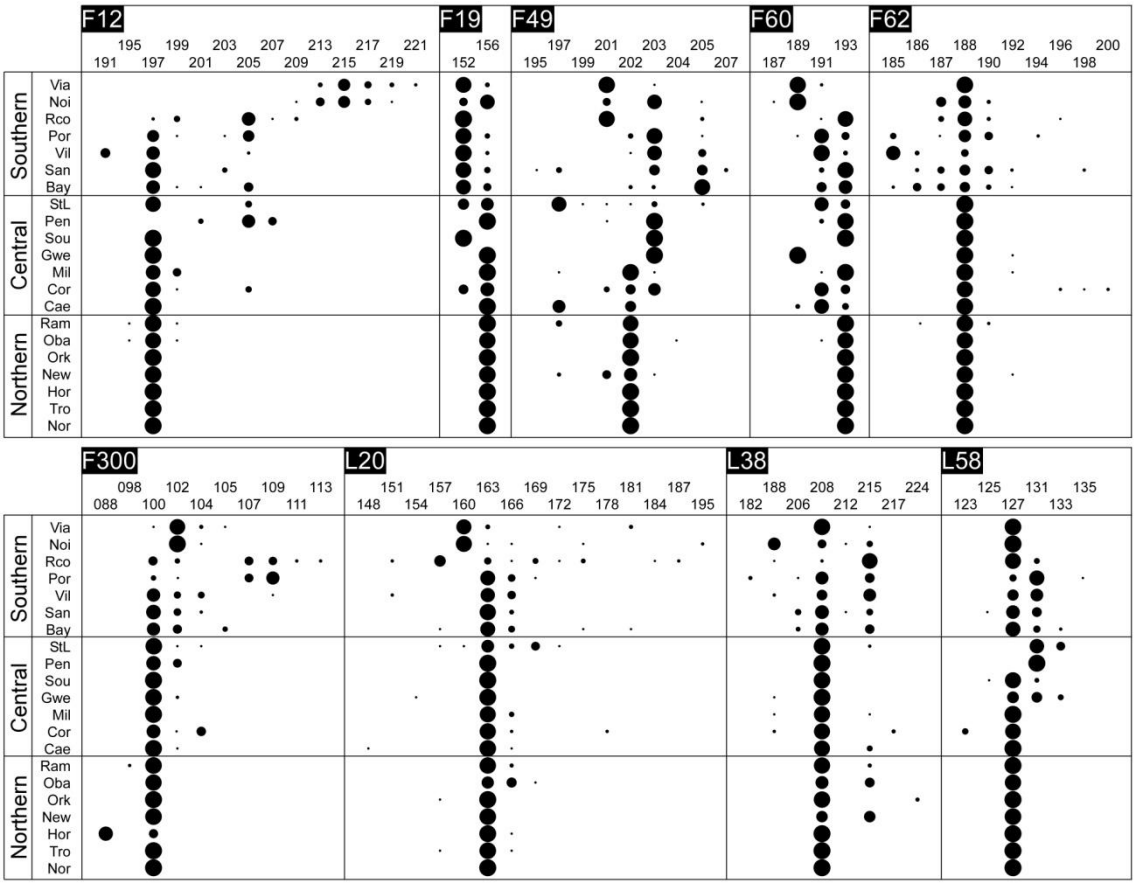


**Figure 3.S1** Microsatellite allele-frequencies in each population of *Fucus ceranoides*. The presence of an allele in a population is indicated by a circle with an area proportional to its frequency. Numbers on top are allele sizes (bp). Population codes are given in Table 3.1. Horizontal lines separate the geographical regions considered: Southern, Central and Northern Europe.

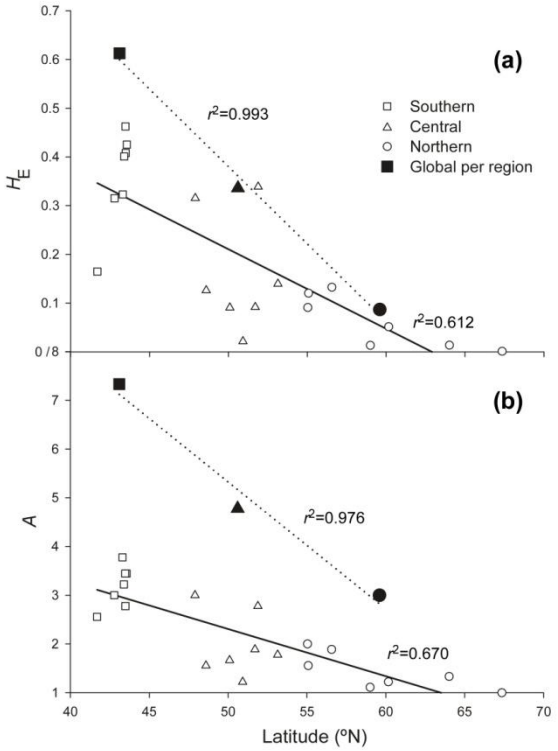


**Figure 3.S2** Latitudinal trends of genetic diversity in *Fucus ceranoides*. **(a)** Gene diversity ($H_E$), and **(b)** mean allelic richness (A). Estimates were computed for each population (open symbols; N=21) and computed for the Southern, Central and Northern regions (filled symbols; N=7 in each region).

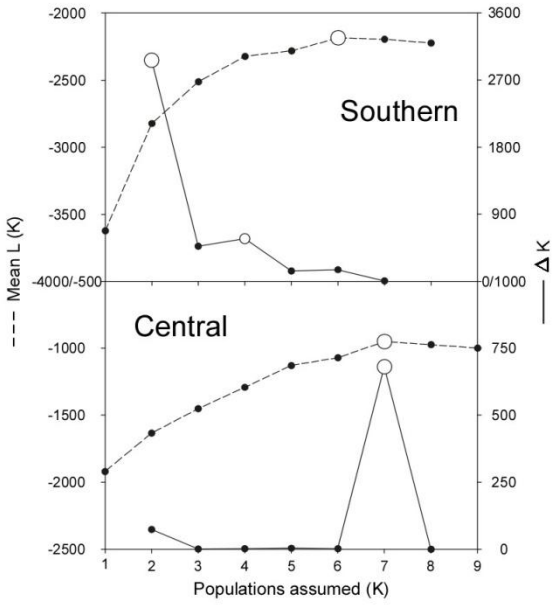


**Figure 3.S3** Genetic subdivision of *Fucus ceranoides* in Southern and Central regions according to STRUCTURE. Five iterations were run for each number of genetic clusters assumed (K). The most probable K (open symbols) were inferred with Pritchard *et al.* (2000; left axis) and Evanno *et al.* (2005; right axis) choice criteria.

## Tables

**Table 3.S1** Primer sequences, PCR conditions and original sources of the 9 microsatellite loci used in this study.

| *Locus* | *Primers 5' → 3'* | *Mg (mM)* | *Cycles (n)* | *$T_a$ (°C)* | *Source* |
|---|---|---|---|---|---|
| F12 | F* : TATGTGTCCGACGACCTGAG<br>R: TGAAGTCAAATGCTTGTTCG | 1.5 | 30-35 | 56 | Coyer *et al*. (2008) |
| F19 | F*: AGGTTTCAACCTGCTTCTGG<br>R: TGCTACATCCAAGAATTGCAG | | | | |
| F49 | F*: TGCTGTAGAAGGCCGAAGTT<br>R: AACGAGTTCGTCGAGTGTCC | | | | |
| F60 | F*: GGGGTTGTTTTCGATAAAAGG<br>R: GCAATCGACCTCGAGAAATC | | | | |
| F62 | F*: GTCTCCACGCCGAAAATTAG<br>R: AGGTTACCACGCAAGCAACT | 1.5 | 30-35 | 56 | Coyer *et al*. (unpublished) |
| F300 | F*: GCATGTGGCGTATAATGACTG<br>R: CCGCTCACAATCCTTCCCTGG | 2.0 | 30<br>10 | 61<br>56 | Wallace *et al*. (2004) |
| L20 | F*: ACTCCATGCTGCGAGACTTC<br>R: CCTCGGTGATCAGCAATCAT | 2.0 | 30-35 | 55 | |
| L38 | F*: TGCTAGCTGCTCTTGTGTGC<br>R: TAACCTGTCGGTCGCAACG | 2.0 | 30-35 | 59 | Engel *et al*. (2003) |
| L58 | F*: AAACGAAAATGGCACAGTGA<br>R: CCTTGCATGTAGGAGGGAAC | 2.0 | 30-35 | 55 | |

* labelled (FAM, HEX, NED) primers.

**Table 3.S2** Pairwise differentiation between the 21 populations of *Fucus ceranoides*. $F_{ST}$ (θ) values are given above diagonal and Jost's $D_{est}$ below diagonal. Non-significant $F_{ST}$ values (1000 permutations) are depicted in bold. Population codes are given in Table 3.1.

| | **VIA** | **NOI** | **RCO** | **POR** | **VIL** | **SAN** | **BAY** | **STL** | **PEN** | **SOU** | **GWE** | **MIL** | **CAE** | **COR** | **RAM** | **OBA** | **ORK** | **NEW** | **HOR** | **TRO** | **NOR** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VIA** | --- | 0.415 | 0.587 | 0.602 | 0.618 | 0.587 | 0.540 | 0.667 | 0.801 | 0.839 | 0.789 | 0.806 | 0.766 | 0.602 | 0.810 | 0.785 | 0.867 | 0.789 | 0.836 | 0.866 | 0.876 |
| **NOI** | 0.089 | --- | 0.581 | 0.540 | 0.546 | 0.538 | 0.498 | 0.623 | 0.688 | 0.757 | 0.645 | 0.712 | 0.675 | 0.533 | 0.716 | 0.683 | 0.769 | 0.690 | 0.742 | 0.768 | 0.778 |
| **RCO** | 0.284 | 0.509 | --- | 0.442 | 0.483 | 0.420 | 0.362 | 0.571 | 0.677 | 0.684 | 0.744 | 0.679 | 0.678 | 0.520 | 0.680 | 0.627 | 0.741 | 0.607 | 0.722 | 0.740 | 0.752 |
| **POR** | 0.475 | 0.514 | 0.301 | --- | 0.188 | 0.286 | 0.291 | 0.351 | 0.473 | 0.529 | 0.549 | 0.616 | 0.548 | 0.326 | 0.613 | 0.592 | 0.667 | 0.590 | 0.641 | 0.666 | 0.675 |
| **VIL** | 0.543 | 0.556 | 0.395 | 0.070 | --- | 0.268 | 0.248 | 0.380 | 0.574 | 0.542 | 0.572 | 0.626 | 0.530 | 0.328 | 0.622 | 0.589 | 0.677 | 0.583 | 0.671 | 0.677 | 0.686 |
| **SAN** | 0.370 | 0.439 | 0.247 | 0.137 | 0.097 | --- | 0.084 | 0.323 | 0.496 | 0.269 | 0.502 | 0.448 | 0.443 | 0.248 | 0.422 | 0.433 | 0.498 | 0.396 | 0.517 | 0.499 | 0.508 |
| **BAY** | 0.359 | 0.460 | 0.234 | 0.129 | 0.123 | 0.024 | --- | 0.343 | 0.512 | 0.427 | 0.531 | 0.450 | 0.419 | 0.256 | 0.438 | 0.424 | 0.505 | 0.408 | 0.511 | 0.504 | 0.514 |
| **STL** | 0.477 | 0.582 | 0.503 | 0.137 | 0.192 | 0.173 | 0.160 | --- | 0.497 | 0.601 | 0.521 | 0.556 | 0.377 | 0.303 | 0.539 | 0.537 | 0.621 | 0.538 | 0.636 | 0.618 | 0.631 |
| **PEN** | 0.527 | 0.430 | 0.352 | 0.187 | 0.291 | 0.170 | 0.242 | 0.141 | --- | 0.799 | 0.674 | 0.722 | 0.719 | 0.498 | 0.729 | 0.722 | 0.818 | 0.713 | 0.796 | 0.816 | 0.832 |
| **SOU** | 0.286 | 0.421 | 0.234 | 0.173 | 0.161 | 0.043 | 0.092 | 0.147 | 0.109 | --- | 0.809 | 0.798 | 0.768 | 0.429 | 0.789 | 0.771 | 0.927 | 0.757 | 0.884 | 0.927 | 0.954 |
| **GWE** | 0.328 | 0.252 | 0.546 | 0.220 | 0.198 | 0.120 | 0.215 | 0.111 | 0.080 | 0.061 | --- | 0.729 | 0.620 | 0.407 | 0.720 | 0.711 | 0.826 | 0.697 | 0.808 | 0.826 | 0.845 |
| **MIL** | 0.410 | 0.440 | 0.349 | 0.362 | 0.346 | 0.099 | 0.137 | 0.131 | 0.112 | 0.051 | 0.073 | --- | 0.480 | 0.348 | 0.060 | 0.187 | 0.126 | 0.277 | 0.466 | 0.098 | 0.142 |
| **CAE** | 0.399 | 0.432 | 0.473 | 0.257 | 0.232 | 0.147 | 0.156 | 0.044 | 0.180 | 0.097 | 0.065 | 0.030 | --- | 0.211 | 0.439 | 0.474 | 0.586 | 0.447 | 0.630 | 0.586 | 0.609 |
| **COR** | 0.350 | 0.313 | 0.395 | 0.146 | 0.160 | 0.117 | 0.104 | 0.059 | 0.131 | 0.060 | 0.057 | 0.053 | 0.024 | --- | 0.339 | 0.366 | 0.409 | 0.345 | 0.437 | 0.408 | 0.420 |
| **RAM** | 0.410 | 0.438 | 0.347 | 0.358 | 0.342 | 0.087 | 0.133 | 0.125 | 0.112 | 0.046 | 0.067 | 0.001 | 0.020 | 0.048 | --- | 0.174 | 0.054 | 0.202 | 0.436 | 0.049 | 0.066 |
| **OBA** | 0.444 | 0.432 | 0.308 | 0.335 | 0.283 | 0.107 | 0.124 | 0.144 | 0.157 | 0.075 | 0.103 | 0.010 | 0.045 | 0.086 | 0.007 | --- | 0.301 | 0.189 | 0.488 | 0.294 | 0.339 |
| **ORK** | 0.410 | 0.445 | 0.358 | 0.383 | 0.365 | 0.096 | 0.149 | 0.135 | 0.113 | 0.046 | 0.067 | 0.001 | 0.026 | 0.052 | 0.001 | 0.008 | --- | 0.352 | 0.633 | **0.025** | **0.044** |
| **NEW** | 0.437 | 0.423 | 0.261 | 0.345 | 0.282 | 0.101 | 0.131 | 0.170 | 0.146 | 0.068 | 0.094 | 0.013 | 0.037 | 0.077 | 0.007 | 0.009 | 0.009 | --- | 0.526 | 0.371 | 0.398 |
| **HOR** | 0.411 | 0.446 | 0.387 | 0.388 | 0.447 | 0.150 | 0.198 | 0.204 | 0.164 | 0.087 | 0.117 | 0.010 | 0.062 | 0.082 | 0.008 | 0.024 | 0.007 | 0.029 | --- | 0.629 | 0.683 |
| **TRO** | 0.410 | 0.446 | 0.356 | 0.382 | 0.361 | 0.097 | 0.149 | 0.132 | 0.112 | 0.046 | 0.067 | 0.001 | 0.027 | 0.052 | 0.000 | 0.008 | 0.000 | 0.009 | 0.007 | --- | **0.007** |
| **NOR** | 0.410 | 0.447 | 0.358 | 0.387 | 0.366 | 0.098 | 0.152 | 0.135 | 0.112 | 0.046 | 0.067 | 0.001 | 0.027 | 0.052 | 0.001 | 0.009 | 0.000 | 0.009 | 0.007 | 0.000 | --- |

**Table 3.S3** Migration rates (individuals per generation) among Southern and Central source (columns) and receptor (lines) populations of *Fucus ceranoides*, estimated with BAYESASS+. Values along the diagonal are the proportions of non-migrant individuals. Putative migration rates >0.1 are highlighted in grey colour. Population codes are given in Table 3.1.

| **Southern** | VIA | NOI | RCO | POR | VIL | SAN | BAY |
|---|---|---|---|---|---|---|---|
| VIA | **0.987** | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 |
| NOI | 0.002 | **0.987** | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 |
| RCO | 0.002 | 0.002 | **0.985** | 0.002 | 0.003 | 0.003 | 0.002 |
| POR | 0.002 | 0.002 | 0.002 | **0.986** | 0.003 | 0.002 | 0.002 |
| VIL | 0.002 | 0.002 | 0.003 | 0.002 | **0.986** | 0.002 | 0.002 |
| SAN | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | **0.987** | 0.002 |
| BAY | 0.006 | 0.006 | 0.006 | 0.006 | 0.006 | 0.292 | **0.679** |

| **Central** | STL | PEN | SOU | GWE | MIL | CAE | COR |
|---|---|---|---|---|---|---|---|
| STL | **0.987** | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 |
| PEN | 0.002 | **0.987** | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 |
| SOU | 0.002 | 0.002 | **0.987** | 0.002 | 0.002 | 0.002 | 0.002 |
| GWE | 0.002 | 0.002 | 0.002 | **0.987** | 0.002 | 0.002 | 0.002 |
| MIL | 0.002 | 0.002 | 0.002 | 0.002 | **0.986** | 0.002 | 0.002 |
| CAE | 0.006 | 0.006 | 0.006 | 0.006 | 0.292 | **0.679** | 0.005 |
| COR | 0.002 | 0.003 | 0.006 | 0.003 | 0.006 | 0.003 | **0.976** |

# **CHAPTER IV.** FINE-SCALE GENETIC BREAKS DRIVEN BY THE COLONIZATION PAST AND PRESENT DENSITY BARRIERS IN THE ESTUARINE SEAWEED *FUCUS CERANOIDES* L.

# Fine-scale genetic breaks driven by the colonization past and present density barriers in the estuarine seaweed *Fucus ceranoides* L.

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**Abstract**

**Background** Factors promoting the emergence of sharp phylogeographic breaks include restricted dispersal, habitat discontinuity, physical barriers, disruptive selection, genetic surfing and secondary contact. Disentangling the role of each in any particular system can be difficult, especially when species are evenly distributed across transition zones and dispersal barriers are not evident. The estuarine seaweed *Fucus ceranoides* provides a good example of highly differentiated populations along its most persistent distributional range at the present rear edge of the species distribution, in NW Iberia. Intrinsic dispersal restrictions are obvious in this species, but have not prevented *F. ceranoides* from vastly expanding its range northwards following the last glaciation, implying that additional factors are responsible for the lack of connectivity between neighbouring southern populations. In this study we analyze 22 consecutive populations of *F. ceranoides* along NW Iberia to investigate the processes generating and maintaining its high levels of regional genetic divergence. **Results** Variation at seven microsatellite loci and at mtDNA spacer sequences was concordant in revealing that

Iberian *F. ceranoides* is composed of three divergent genetic clusters displaying nearly disjunct geographical distributions. Within each genetic cluster, population structure was also pervasive, although shallower. Haplotypic diversity was higher in the W sector, and very low in the N sector. MtDNA variation was consistent with the spatial expansions of phylogroups A and C. **Conclusions** The deep divergence between sectors coupled with the lack of support for a role of oceanographic barriers in defining the location of breaks suggested 1) that the parapatric genetic sectors result from the regional reassembly of formerly vicariant sub-populations, and 2) that the genetic discontinuities at secondary contact zones (and elsewhere) are maintained despite normal migration rates. We conclude that colonization and immigration, as sources of gene-flow, have very different genetic effects. Migration between established populations is effectively too low to prevent their differentiation by drift or to smooth historical differences inherited from the colonization process. *F. ceranoides*, but possibly low-dispersal species in general, appear to be unified to a large extent by historical, non-equilibrium processes of extinction and colonization, rather than by modern patterns of gene flow.

**Keywords** Fine-scale phylogeography, *Fucus ceranoides*, genetic sectors, non-equilibrium divergence, density-barrier effects, secondary contact zone

## Introduction

Marine ecosystems have historically been considered to be relatively open, with populations demographically and genetically connected over broad spatial scales. In a range of coastal taxa, however, recent molecular surveys have consistently revealed considerable phylogeographical and population genetic structure, often at seemingly

small spatial scales, indicating that connectivity is frequently much lower than previously assumed. Examples include a variety of species lacking planktonic dispersive stages and/or exhibiting particularly strict ecological requirements, such as intertidal fucalean and kelp seaweeds (Hoarau *et al.* 2007; Tellier *et al.* 2009; Cheang *et al.* 2010; Fraser *et al.* 2010; Coyer *et al.* 2011), seagrasses (Arnaud-Haond *et al.* 2007; Alberto *et al.* 2008), direct-developing invertebrates and fish (Planes *et al.* 2001; Duvernell *et al.* 2008; Xavier *et al.* 2009; Hurtado *et al.* 2010), high-intertidal rock-pool invertebrates (Willett & Ladner 2009), and many estuarine organisms (Dawson *et al.* 2001; Diekmann *et al.* 2005; Kelly *et al.* 2006; Teske *et al.* 2006; Remerie *et al.* 2009; Neiva *et al.* 2010). In such species, shallow genetic discontinuities can be common due to intrinsic life-history and habitat constraints to dispersal. Often, however, they also display deeper genealogical splits that distinguish regional sets of populations across their ranges. Such nested patterns of phylogeographical structure can result from a number of factors and are frequently harder to interpret, especially when species are evenly distributed across transition zones.

Vicariance is usually invoked as the main driver of (neutral) genetic divergence. Extrinsic barriers to gene-flow are generally less obvious (or absolute) in marine compared to terrestrial landscapes (Thorrold 2006; Patarnello *et al.* 2007), but circulation patterns, coastline topography and habitat discontinuities have all been shown to potentially represent effective barriers to the exchange of individuals between adjacent marine regions (Barber *et al.* 2002; Sotka *et al.* 2004; Cowen & Sponaugle 2009; Galarza *et al.* 2009). Complex variations in habitat availability and connectivity, resulting from the Pleistocene oscillations in sea levels and surface temperatures, are also known to have produced ancient population subdivisions (and differentiation) in many coastal organisms (Barber *et al.* 2000; Alberto *et al.* 2008; Hobbs *et al.* 2009).

Within a species, disjunct distribution of divergent genetic lineages provides strong indication for the occurrence of such vicariant processes.

Inferring the existence of a particular dispersal barrier from molecular data may not be straightforward though (Marko & Hart 2011a). In species with short dispersal range, discontinuities in individual gene trees (mostly derived from organelle markers) readily arise haphazardly within continuously distributed species simply as a consequence of idiosyncratic lineage sorting (Irwin 2002; Kuo & Avise 2005). Similarly, genetic drift during spatial expansions (Excoffier & Ray 2008) or disruptive selection (Rawson & Burton 2006) can also result in the geographic segregation of organelle lineages across a species range even in the face of dispersal. In general, long-term isolation can only be confidently assumed when spatially concordant patterns across multiple unlinked loci are found (Avise 2000; Kuo & Avise 2005).

Disentangling historical from modern constraints to dispersal may also be problematic. Phylogeographical breaks and contemporary oceanographic barriers (or biogeographical transition zones) are often mismatched in marine restricted dispersers (Pelc *et al.* 2009). Historical patterns of isolation and colonization in these organisms explain population structure better than more recent factors affecting gene-flow. Phylogeographical breaks may also develop where formerly vicariant sub-populations have reassembled. The Iberian peninsula is a good example where diverse taxa such as trees (Bucci *et al.* 2007), amphibians (Martinez-Solano *et al.* 2006; Gonçalves *et al.* 2009), reptiles (Godinho *et al.* 2008; Pinho *et al.* 2008) and pond-dwelling invertebrates (Gómez *et al.* 2007b; Korn *et al.* 2010) are sub-divided into well defined, mostly parapatric genetic sectors that presumably formed during expansions from disjunct refugia. The temporal persistence of genetic discontinuities across marine secondary contact zones have also been demonstrated in several species (Hobbs *et al.* 2009).

However, insight into the processes preventing steady genetic homogenization of divergent but contacting gene-pools requires finer scale genetic sampling than is common in most studies [but see (Van Herwerden & Doherty 2006; Alberto *et al.* 2008; Willett & Ladner 2009; Fraser *et al.* 2010; Hurtado *et al.* 2010; Tellier *et al.* 2011)].

Virtually all coastal organisms have some potential to disperse and colonize new habitats, as the extensive post-glacial range shifts of many demonstrate. Thus, migration would also be expected to occur between fully established populations, including between divergent populations in relatively close proximity. In this sense, the persistence of fine-scale genetic differentiation in the absence of dispersal barriers seems paradoxical. In restricted dispersers, however, colonization and immigration, as sources of gene-flow, may have very different genetic effects. During expansions into vacant habitats, the original colonists can grow exponentially and contribute disproportionately to the genetic composition of the establishing population. Contrastingly, once the habitat patch is filled, demographic stability and increased competition can considerably reduce the impact of subsequent immigrants. In addition, if there is a gross disparity between the number of residents and immigrants, a common situation in low dispersal species, foreign genotypes introduced in a population will *a priori* be rare and have low probability of random increase due to drift alone. In other words, established populations themselves can create a density-barrier effect buffering local changes in allele frequencies and delaying the spatial advance of genes within previously colonized areas (despite immigration).

At broad geographical scales, such an effect has been invoked to explain the persistence of genetic homogeneity in recolonized areas (Hewitt 2000), the asymmetrical introgression of genes from established to spatially expanding species (Currat *et al.* 2008), or the lack of gene-flow between former refugial areas that are

currently connected by intermediate populations (Petit *et al.* 2003). When effective migration rates are low, patterns of non-equilibrium divergence resulting from founder and density-barrier effects can occur at much smaller spatial scales (Boileau *et al.* 1992; De Meester *et al.* 2002). In this study, we report one remarkable case of such non-equilibrium divergence in the estuarine seaweed *Fucus ceranoides*, where steep genetic discontinuities are preserved despite the absence of obvious barriers to dispersal.

*Fucus ceranoides* L. (horned wrack) is a perennial, dioecious seaweed restricted to estuarine environments across much of the Northeast Atlantic. Populations of *F. ceranoides* from NW Iberia, at the rear edge of the species distribution, form three highly divergent genetic clusters according to both mtDNA and microsatellite markers(Neiva *et al.* 2010; Neiva *et al. in press*). Despite their relatively close proximity (~150 km), fixed genetic differences at this scale suggest that the historical and recurrent processes contributing to their differentiation are weakly counteracted by on-going gene-flow. The poor dispersal ability of *F. ceranoides* certainly plays a role; fucoid algae lack planktonic dispersive stages and therefore *F. ceranoides* individuals typically complete their entire life-cycle within the discrete, isolated patches of the estuarine habitat they inhabit. Still, an important question remains unanswered concerning the nature and stability of genetic divergence in this system. Like in many other seaweeds, non-local (inter-estuarine) dispersal can be mediated by rafting of detached, reproductive individuals (Norton 1992; McKenzie & Bellgrove 2008). Such dispersal by drifting thalli was likely responsible for the extensive post-glacial expansion of *F. ceranoides* into Northern Europe, including the distant colonization of Norway (across the North Sea) and Iceland (Neiva *et al.* 2010; Neiva *et al. in press*). If *F. ceranoides* managed to expand its range more than 15 degrees in latitude since the Last Glacial Maximum [LGM, ~20.000 ka before present (BP)], dispersal restrictions

cannot account, at least as the sole factor, for the apparent lack of population connectivity along the much narrower NW Iberian coastline.

This study aims to understand this fundamental issue in the evolutionary ecology of populations, the apparently contradicting evidence for large scale dispersal mediating vast (re)colonisations concurrently with persistent, fine scale genetic discontinuities in older refugial regions. The specific question is whether such discontinuities arise and persist due to long-lasting dispersal barriers, or simply reflect resilient non-equilibrium conditions inherited from a complex demographic past. To address this question, in this study both mtDNA sequence and microsatellite genotypic data are employed to investigate the fine-scale distribution of genetic variation in *F. ceranoides* from NW Iberia. This region was sampled at the finest scale of resolution achievable – a complete set of neighbouring estuaries - which was the scale over which gene-flow was more likely to be detected. We were particularly interested in the biogeographic context and the demographic processes contributing to the formation and integrity of stable genetic sectors in NW Iberian *F. ceranoides*.

## Material and Methods

*Sampling, DNA isolation, sequencing and genotyping*

The "core" populations of *F. ceranoides* used in this study were collected in the estuaries of all major rivers between Vigo (VIG, SW Galicia) and Navia (NAV, W Asturias), in NW Iberia (N=22; Table 4.1; Fig. 4.1). This corresponded approximately to an array of discrete but neighbouring populations with an average proximity of about 33 (±17) km. Four additional populations Iberian that are not contiguous to this "core" population set were included in some analyses, namely VIA (northern Portugal), VIL (eastern Asturias), SAN (Cantabria) and BAY (southern France). These and also 3

"core" populations – NOI, RCO and POR – were previously analysed by Neiva *et al.* (Neiva *et al.* 2010; Neiva *et al. in press*). All collection sites typically contained monospecific belts of *F. ceranoides* attached to hard substrata and were exposed to steep salinity fluctuations throughout the tidal cycle. At each site, 5-10 cm tips of apical vegetative tissue was excised from 16 individuals sampled along a 100-200m linear transect or random walk; tissue samples were individually stored dehydrated in silica-gel crystals until DNA extraction. To keep sample sizes constant, a random subsample of 16 individuals was used from the previously analysed populations.

Genomic DNA was extracted from approximately 10 mg dried tissue using the Nucleospin® Multi-96 plant kit (Macherey-Nagel Duren, Germany), according to the manufacturer's protocol. Individuals were sequenced for the mitochondrial 23S/trnK intergenic spacer (mtIGS, (Neiva *et al.* 2010)), and genotyped for 7 microsatellite loci developed for congeners (Engel *et al.* 2003; Wallace *et al.* 2004; Coyer *et al.* 2009) that had shown polymorphism in Iberian *F. ceranoides* (Neiva *et al. in press*). Primer sequences and amplification details were the same as in Neiva *et al*. (Neiva *et al.* 2010; Neiva *et al. in press*). Amplified fragments were run in an ABI PRISM 3130xl automated capillary sequencer (Applied Biosystems, CCMAR Portugal). MtDNA sequences were aligned, proofread and edited in GENEIOUS 3.8 (Drummond *et al.* 2010). Microsatellite alleles were manually scored in STRAND (Toonen & Hughes 2001) using the 350 ROX™ size standard (Applied Biosystems).

*Genetic structure*

The geographic distribution of the mtDNA variation was mapped and the genealogic relationships of haplotypes were inferred using the median-joining algorithm implemented in Network 4.5 (Bandelt *et al.* 1999). A phylogenetic tree for the mtDNA

sequences was reconstructed with MrBayes (Ronquist & Huelsenbeck 2003) using the best-fit model of nucleotide substitution. Among the 88 models evaluated in jModeltest (Guindon & Gascuel 2003; Posada 2008), the HKY + G model was selected based on the Akaike information ranking. Two parallel Metropolis-coupled Markov chain Monte Carlo searches, each with four chains, were run for 2 x $10^6$ generations, sampling every 100 generations. The number of substitution rates (Nst = 2) and among-site rate variation (Rates = Gamma) were set according to the substitution model selected, leaving the remaining options as default. $10^5$ generations (1000 trees) were discarded as burn-in, and the remaining 38000 used to produce 50% majority-rule consensus trees and to calculate branch posterior probabilities.

Nucleotide ($\pi_{hap}$) and haplotypic ($H_{hap}$) diversity within populations and inferred mtIGS phylogroups (see RESULTS) were calculated with DNASP 5.10 (Librado & Rozas 2009). Summary statistics of the microsatellite genetic diversity, including microsatellite allele frequencies, mean allelic richness ($A$), Nei's gene diversity ($H_E$), observed heterozygosity ($H_O$) and inbreeding coefficients ($F_{IS}$), were calculated with GENETIX 4.05 (Belkhir *et al.* 1996-2004). The partitioning of genetic variation between and among the mtIGS sectors was examined with molecular analyses of variance (AMOVA) in ARLEQUIN 3.1 (Schneider *et al.* 2000). The significance ($P >$ 0.05) of the fixation indices was calculated after 1000 permutations of individuals within sectors. For each phylogroup, the occurrence of recent spatial expansions (assuming constant deme size) was tested with ARLEQUIN 3.1 (Schneider *et al.* 2000), fitting the implemented model to the observed mismatch distribution. Significance was assessed with 1000 permutations.

The microsatellite population structure was assessed with both individual (genotype based) and population (allele-frequency based) approaches. First, the degree

of congruence between the mtIGS structure/phylogeny and its nuclear background was visually inspected with the factorial correspondence analysis (FCA) implemented in GENETIX 4.05 (Belkhir *et al.* 1996-2004). Population genetic structure was further examined with a Bayesian, model-based genetic admixture analysis implemented in STRUCTURE 2.3 (Pritchard *et al.* 2000; Falush *et al.* 2003). Individuals were combined into one dataset for analysis, without any *a priori* population assignments and admixture was allowed. Each number of assumed populations (*K*, set sequentially from 1 to 14) was ran five times using a burn-in of 200000 iterations and a run-length of 1000000 iterations, which was determined to be sufficient to have consistent results. The "true" number of *K* was inferred both from the posterior probability of the data, hereafter referred to as *L(K)*, and following the $\Delta K$ choice criterion (Evanno *et al.* 2005), better suited to detect heterogeneous patterns of dispersal or co-ancestry.

Pairwise $F_{ST}$ ($\theta$; (Weir & Cockerham 1984)) was estimated with GENETIX 4.05 (Belkhir *et al.* 1996-2004) and pairwise D ($D_{est}$; (Jost 2008)) was estimated with SMOGD 1.25 (Crawford 2010). Isolation by distance (IBD) was evaluated for full and sub data-sets using reduced major axis regressions of pairwise estimates of population's genetic differentiation against minimum marine distance, as measured in Google Earth 5.1. The statistical significance of the genetic and geographic associations was assessed with Mantel tests (1000 randomizations, $P< 0.05$) in IBDWS (Jensen *et al.* 2005).

## Results

### *MtIGS phylogeography*

A total of 51 mtIGS haplotypes (GenBank: JN084346-96) were identified in the 352 individuals of *F. ceranoides* belonging to the 22 "core" populations. The median-joining network revealed three mtIGS lineages displaying nearly disjunct geographic

distributions (Table 4.1 and Fig. 4.1). Each phylogroup was defined by one interior and widespread haplotype. Phylogroup A, composed by haplotypes *A1* and 30 related ones, was present from VIG to CAM (Western sector, W), and further south in VIA. Phylogroup B, composed by *B1* and 10 related haplotypes, was distributed from ANL to CED (North-Western sector, NW), although a few B haplotypes were also detected in ORT and BAR. Finally, phylogroup C, composed by *C1* and 8 related haplotypes, was exclusively found from ORT eastwards to NAV (Northern sector, N), and further east in the populations of VIL, SAN and BAY. Several peripheral populations were geographically closer to populations across the phylogeographic breaks than they were to their nearest population within the same sector. For instance, ANL (NW sector) is geographically closer to CAM (W sector; ~34km) than to RCO (NW sector, ~70km), and the distance between CED (NW sector) and ORT (N sector; ~36,5 km) is smaller than between CED and FER (~58km; both NW sector).

Globally, only the three dominant haplotypes (*A1*, *B1* and *C1*) plus two derived ones (*A2*, *B2*) were shared among at least two populations. The remaining 46 haplotypes were population-specific and among these, 12 represented non-singleton variants. Many W and NW populations harboured private haplotypes in relatively high frequencies. This pattern was apparent even in populations located inside the same drainage systems, such as UMI and ULL (Ria de Arousa, W sector), or RCO, BET, ARE and FER (Artabro Gulf, NW sector). $H_{hap}$ was high in the W ($H_{hap}$=0.717) and NW ($H_{hap}$=0.671) sectors due to the presence of most of the local haplotype radiations, but $\pi_{hap}$ was considerably higher in the former. Contrastingly, $H_{hap}$ was very low in the N sector ($H_{hap}$=0.166), but further east the populations of VIL and SAN possessed private, C1-derived haplotypes in relatively high frequencies.

**Table 4.1** Genetic diversity of *Fucus ceranoides* within sampling sites and inferred genetic sectors. Mean allelic richness (A), Nei's gene diversity ($H_E$), observed heterozygosity ($H_o$) and multi-locus inbreeding coefficient ($F_{IS}$,) were estimated from for the microsatellite data-set; Haplotypic richness ($N_{hap}$), haplotypic diversity ($H_{hap}$) and nucleotide diversity ($\pi_{hap}$) are based on the mtIGS data-set. The mtIGS lineages and haplotypes are listed for each population (coded as in Fig. 4.1). Absolute frequencies of haplotypes are in parenthesis (if N>1). *GenBank assessions of the private haplotypes of the populations of VIA, VIL, SAN and BAY (Neiva *et al*., 2010)

| River (Ria), Village | Code | N | Microsatellites | | | | MtIGS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *A* | $H_E$ | $H_O$ | $F_{IS}$ | Lineage | Haplotypes | $N_{hAP}$ | $H_{hAP}$ ($10^{-3}$) | $\Pi_{hAP}$ ($10^{-5}$) |
| Lima, Viana do Castelo | VIA | 16 | 2.43 | 0.154 | 0.143 | 0.077 | A | A1(14), GQ385159*, GQ385160* | 3 | 242 | 50 |
| **Western sector** | **W** | **128** | **9.00** | **0.457** | **0.257** | | **A** | **A1-A31** | **31** | **717** | **351** |
| Verdugo, (Ria de Vigo), Arcade | VIG | 16 | 3.57 | 0.223 | 0.223 | -0.001 | A | A1(11), A3(3), A4, A5 | 4 | 517 | 116 |
| Lérez (Ria de Pontevedra), Pontevedra | PON | 16 | 2.29 | 0.174 | 0.161 | 0.080 | A | A1(13), A6, A7, A8 | 4 | 242 | 50 |
| Umia (Ria de Arousa), Cambados | UMI | 16 | 2,86 | 0.225 | 0.174 | 0.231* | A | A1, A2(5), A9(3), A10 (2), A11, A12(2), A13, A14 | 8 | 875 | 580 |
| Ulla (Ria de Arousa), Catoira | ULL | 16 | 2,71 | 0.144 | 0.146 | -0.012 | A | A1, A2(9), A15(2), A16, A17, A18, A19 | 7 | 692 | 267 |
| Tabra/Tambre (Ria de Muros e Noia), Noia | NOI | 16 | 3.00 | 0.357 | 0.370 | -0.039 | A | A1(11), A20(5) | 2 | 458 | 92 |
| Xallas (Ria de Córcubion), Ézaro | XAL | 16 | 3.71 | 0.478 | 0.369 | 0.234* | A | A1 (5), A21(9), A22, A23 | 4 | 617 | 151 |
| Castro, Lires | LIR | 16 | 3.29 | 0.447 | 0.324 | 0.282* | A | A1 (11), A24, A25, A26, A27, A28 | 6 | 350 | 104 |
| Grande (Ria das Camariñas), Ponte do Porto | CAM | 16 | 3.43 | 0.430 | 0.279 | 0.360* | A | A1(10), A29(4), A30, A31 | 4 | 575 | 151 |
| **Northwestern sector** | **NW** | **96** | **6.57** | **0.526** | **0.327** | | **B** | **B1-B11** | **11** | **671** | **186** |
| Anllóns (Ria de Corme e Laxe), Ponteceso | ANL | 16 | 3.57 | 0.482 | 0.482 | 0.001 | B | B2 (13), B3(2), B4 | 3 | 342 | 72 |
| Mero (Ria de A Coruña), O Temple | RCO | 16 | 4.00 | 0.355 | 0.304 | 0.150* | B | B1 (10), B5(3), B6(2), B7 | 4 | 592 | 203 |
| Mendo/Mandeo (Ria de Betanzos), Betanzos | BET | 16 | 3.14 | 0.364 | 0.265 | 0.277* | B | B1(7), B8 (9) | 2 | 525 | 106 |
| Eume (Ria de Ares), Pontedeume | ARE | 16 | 4.00 | 0.408 | 0.342 | 0.166* | B | B1(14), B9, B10 | 3 | 242 | 50 |
| Xuvia (Ria do Ferrol), Neda | FER | 16 | 3.71 | 0.458 | 0.368 | 0.203* | B | B1(16) | 1 | - | - |
| Ferrerias (Ria de Cedeira) | CED | 16 | 3.00 | 0.398 | 0.200 | 0.505* | B | B2(15), B11 | 2 | 125 | 25 |
| **Northern sector** | **N** | **128** | **6.29** | **0.553** | **0.441** | | **B,C** | **B1, C1-C9** | **10** | **166** | **144** |
| Mera (Ria de Ortigueira), Ponte de Mera | ORT | 16 | 3.00 | 0.468 | 0.362 | 0.232* | B, C | B1(2), C1(12), C2, C9 | 4 | 442 | 323 |
| Sor (Ria de Barquero), Poceira | BAR | 16 | 3.14 | 0.563 | 0.411 | 0.277* | B, C | B1, C1(14), C3 | 3 | 133 | 113 |
| Landro (Ria de Viveiros), Viveiros | VIV | 16 | 3.57 | 0.484 | 0.473 | 0.022 | C | C1(15), C4 | 2 | 125 | 25 |
| Ouro | FAZ | 16 | 3.43 | 0.497 | 0.500 | -0.007 | C | C1(15), C5 | 2 | 125 | 25 |
| Masma (Ria da Foz) | FOZ | 16 | 3.57 | 0.507 | 0.427 | 0.162* | C | C1(16) | 1 | - | - |
| Eo (Ria de Ribadeo), Vegadeo | VEG | 16 | 3.71 | 0.416 | 0.414 | 0.005 | C | C1(15), C6 | 2 | 125 | 25 |
| Porcia | POR | 16 | 3.00 | 0.463 | 0.527 | -0.144 | C | C1(15), C7 | 2 | 125 | 25 |
| Navia (Ria de Navia), Navia | NAV | 16 | 3.71 | 0.464 | 0.413 | 0.114 | C | C1(15), C8 | 2 | 125 | 25 |
| Valdediós (Ria de Villaviciosa) | VIL | 16 | 3.00 | 0.464 | 0.464 | 0.000 | C | C1(10), GQ385170*(5), GQ385171* | 3 | 542 | 117 |
| Asón (Ria de Santoña), Colindres | SAN | 16 | 3.14 | 0.410 | 0.411 | -0.003 | C | GQ385172*(9), GQ385173*(7) | 2 | 525 | 105 |
| Adour, Bayonne | BAY | 16 | 3.43 | 0.486 | 0.446 | 0.083 | C | C1(13), GQ385174*(2), GQ385175* | 3 | 342 | 72 |

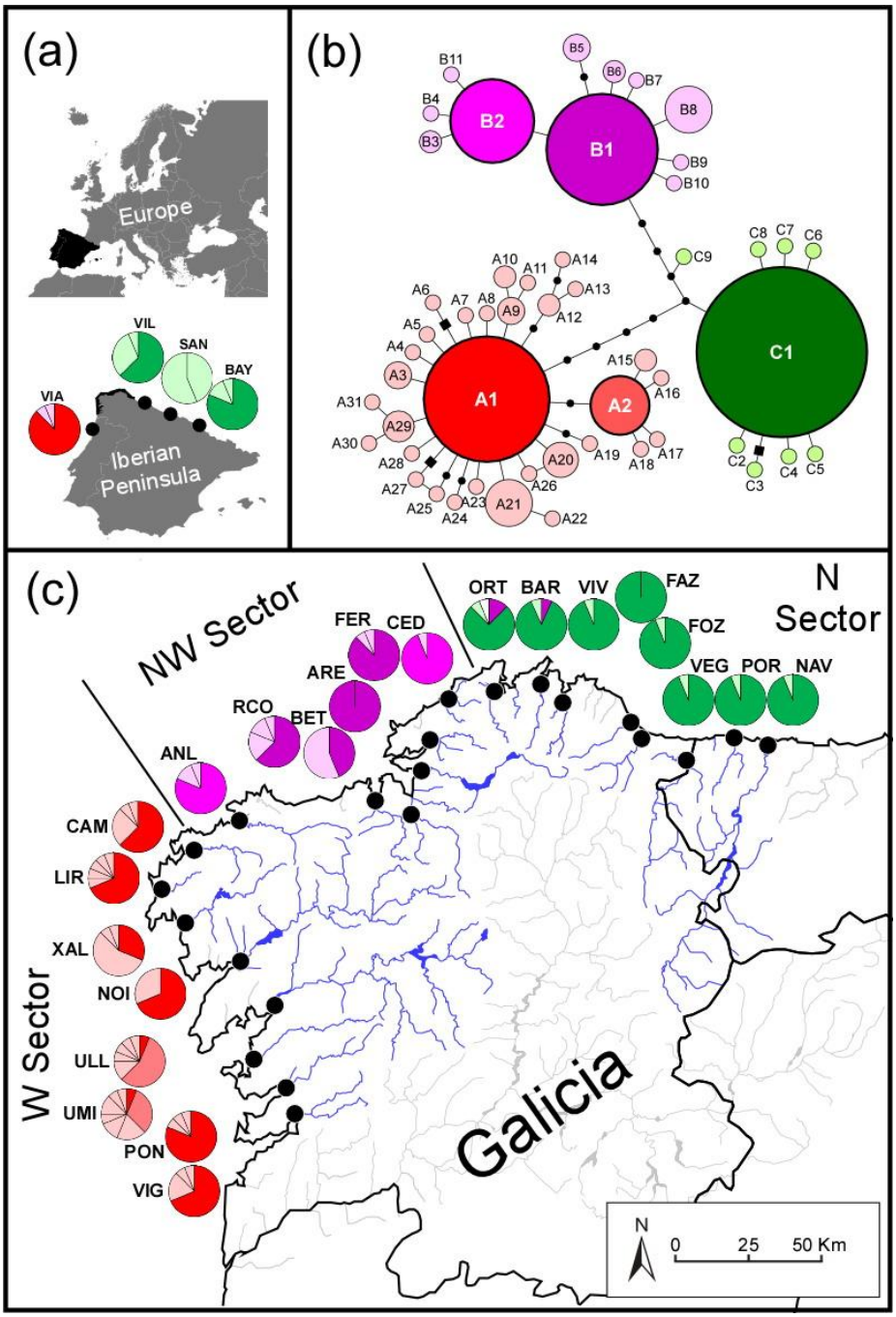


**Figure 4.1** Genealogy and distribution of the mtIGS haplotypes of *Fucus ceranoides* from NW Iberia. **(a)** Location of the study area (in black) in relation to Europe and the Iberian Peninsula. The geographical location and mtIGS lineages present in four Iberian populations previously analysed in Neiva *et al*. (2010) are also shown. **(b)** MtIGS parsimony networks of NW Iberian haplotypes. Sampled haplotypes are represented by circles sized to their frequency and black dots represent inferred, unsampled haplotypes. Links represent a single nucleotide change and black squares represent small indels. Inferred phylogroups are labelled by colour and letter (A-Red; B-Purple; C-Green). Shared and private haplotypes are depicted in bright and pale colour intensity, respectively. (c) Location of sampling sites, delimitation of the phylogeographic sectors considered (Western- W; Northwestern- NW; Northern- N). Pie charts depict haplotype frequencies at each site (see Table 4.1 for haplotype ID's, haplotypes are coloured as in **(b)**.

The results of the AMOVAs showed that the sectors considered accounted for about 83% of the molecular variance of NW Iberian *F. ceranoides* (Table 4.2). Within the W, NW and N sectors, 32%, 63% and 2% of the molecular variance was accounted for by the molecular differences among respective populations.

**Table 4.2** Analyses of molecular variance (AMOVA) between and among NW Iberian genetic sectors of *Fucus ceranoides*. *P* values are based on 1000 permutations.

| ***Analysis*** | ***N*** | ***Level*** | ***d.f.*** | ***Variance (%)*** | ***Fixation indices*** |
|---|---|---|---|---|---|
| 3 sectors | 352 | Among groups<br>Among populations within groups<br>Within populations | 2<br>19<br>330 | 83.07<br>5.55<br>11.38 | $\Phi_{CT}$= 0.831*<br>$\Phi_{SC}$= 0.328* |
| W sector | 128 | Among populations<br>Within populations | 7<br>120 | 32.01<br>67.99 | $\Phi_{ST}$= 0.320* |
| NW sector | 96 | Among populations<br>Within populations | 5<br>90 | 63.44<br>36.56 | $\Phi_{ST}$= 0.634* |
| N sector | 128 | Among populations<br>Within populations | 7<br>120 | 2.24<br>97.76 | $\Phi_{ST}$= 0.023 |

The unrooted Bayesian tree revealed an earlier divergence of phylogroup A (W sector) from phylogroups B and C (NW and N sectors), which split from a more recent common ancestor (Fig. 4.2a).

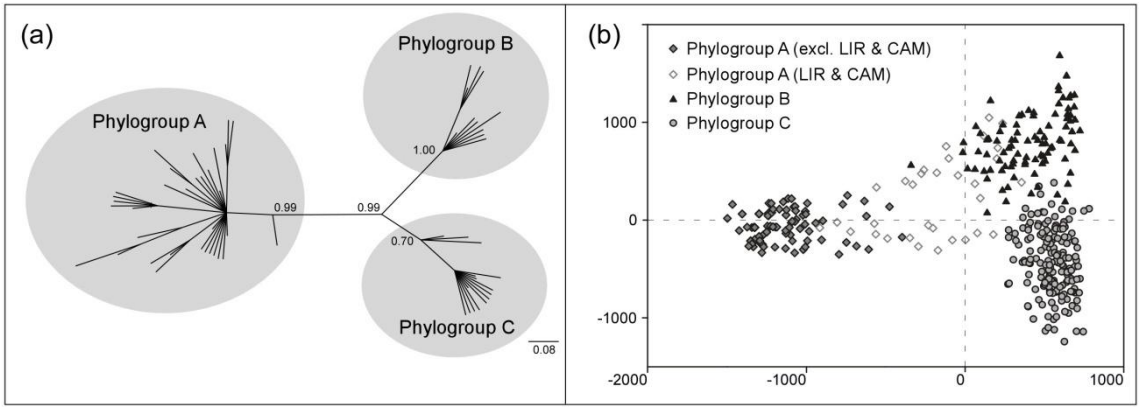


**Figure 4.2** MtIGS phylogeny and mtDNA/nDNA congruence in Iberian *Fucus ceranoides*. **(a)** 50% majority-rule consensus (unrooted) tree of mtIGS haplotypes of *F. ceranoides*. Numbers above the branches are Bayesian posterior probabilities (> 0.70). Inferred phylogroups are highlighted in grey. **(b)** FCA plot based on individual multilocus genotypes. Individuals are labelled according to their mtIGS lineage. Note the correspondence between the mtIGS phylogeny and the nuclear population structure. The individuals from VIA, VIL, SAN and BAY were included in both analyses.

The mismatch distributions did not reject the spatial expansion of phylogroups A (*P*=0.957) and C (*P*=0.750), but failed to support the expansion of phylogroup B (*P*= 0.009; Fig. 4.3).

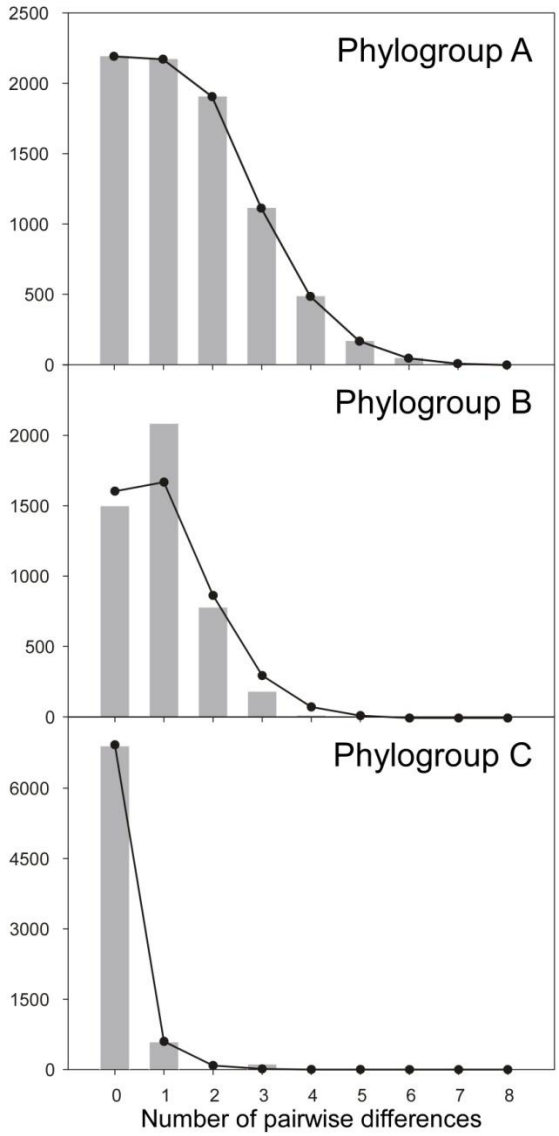


**Figure 4.3** Mismatch distributions of the mtIGS phylogroups A, B and C of *Fucus ceranoides*. The grey bars and the solid lines depict the observed and expected (under the spatial expansion model) values, respectively.

*Microsatellite population structure*

The seven microsatellite loci revealed a total of 76 alleles in the 352 "core" individuals genotyped (6-23 per locus), although 42% of these had global frequencies below 0.01. $H_E$ was rather variable among populations, ranging from 0.144 (ULL; W sector) to 0.563 (BAR, N Sector) (Table 4.1). Approximately half of the populations showed significant heterozygote deficiencies, a possible artefact of estimates based on small sample sizes [such deviations were not found in larger (N=24) samples analyzed in Neiva *et al.* (Neiva *et al. in press*). Among sectors, $H_E$ and $H_{hap}$ were not correlated (Fig. 4.S1a and 4.S1b). The W sector had the lowest (yet most variable) $H_E$ (0.457) and the highest $H_{hap}$ (0.717), whereas the N sector had the highest $H_E$ (0.553) despite very low $H_{hap}$ (0.166). The NW sector showed intermediate levels of diversity for both markers. FST ranged from 0.021 (VIG vs. UMI) to 0.685 (PON vs. CED), whereas $D_{est}$ ranged from <0.001 (VIG vs. PON) to 0.879 (PON vs. ANL) (Table 4.S1). Within sectors, pairwise differentiation of populations was of the same order of magnitude, but more variable within the W sector (Fig. 4.S1c).

The microsatellite genotypic clusters recovered with the FCA showed a remarkable correspondence with the mtIGS phylogroups (Fig. 4.2b). The most obvious exceptions were the populations of LIR and CAM, both belonging to the W sector, whose genotypes appeared intermediate between W and NW and W and N sectors, respectively. Excluding these admixed populations, the W populations formed the most differentiated cluster among the three, as in the phylogenetic tree (Fig. 4.2a). The STRUCTURE analyses showed a similar picture (Fig. 4.4). Based on the ΔK ad-hoc criterion (Evanno *et al.* 2005) the highest hierarchical level of genetic sub-division of *F. ceranoides* occurred between the W sector and the NW and N sectors (K=2; Fig. 4.S2).

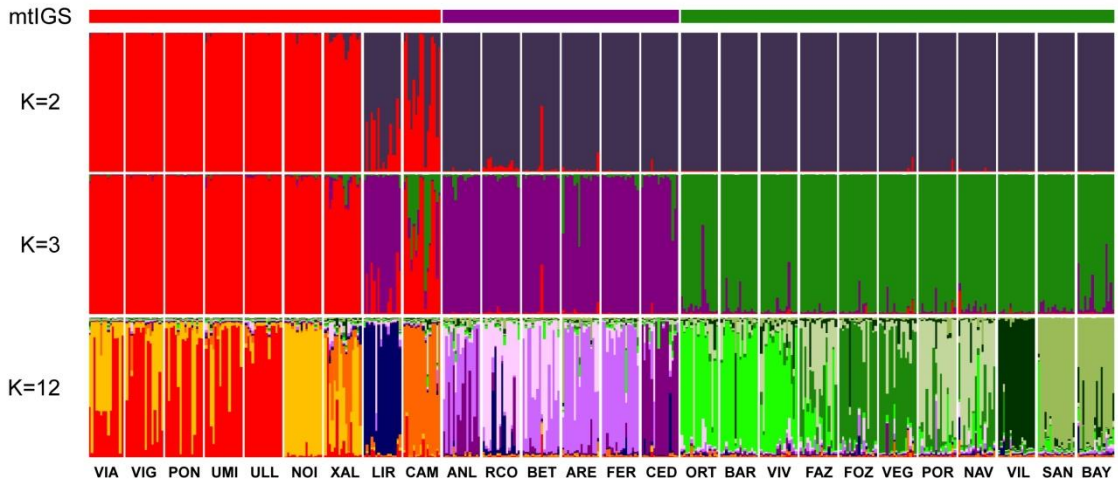


**Figure 4.4** Genetic subdivisions of Iberian *Fucus ceranoides* based on STRUCTURE. Shown are the proportions of individual multilocus genotypes assigned to each of K virtual clusters, as illustrated by the different colours. The individuals from VIA, VIL, SAN and BAY were also included. Population codes are given in Table 4.1.

Further subdivision of genotypes into W, NW and N sectors (K=3) represented a weaker, but nevertheless significant, level of population subdivision. Again, the individuals of LIR and CAM showed variable degree of admixture between the W and the NW and N sectors, respectively. Within these 3 major groups some sub-structuring was also evident (Fig. 4.4). Iberian *F. ceranoides* could be subdivided into a maximum of 12 (stable) genetic clusters (K=12), corresponding to smaller, less resolved geographic regions. Some admixture (or mixed-ancestry) between neighbouring clusters was pervasive, but also apparent in a few, well separated population pairs (e.g. LIR & RCO, ANL & CED).

A significant IBD pattern was detected in the whole NW Iberian region ($P$=0.001), as well as in the W ($P$=0.009) and N ($P$=0.009) sectors (Fig. 4.5). In the W, however, the relationship was lost when the admixed populations of LIR and CAM were removed from the analysis ($P$=0.135; data not shown).

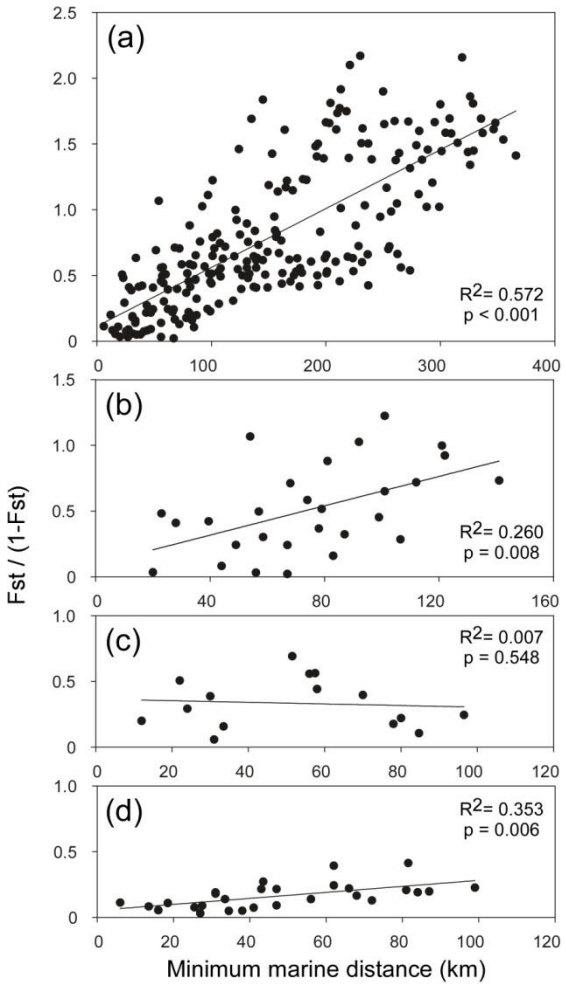


**Figure 4.5** Isolation by distance in *Fucus ceranoides* from NW Iberia. Estimates of pairwise differentiation ($F_{ST}$/1-$F_{ST}$) are plotted against geographic distance for **(a)** NW Iberia **(b)** W sector, **(c)** NW sector and **(d)** N sector. The regressions are: y = 0,0045x + 0,1134), y = 0,0056x + 0,0934, y = -0,0006x + 0,3652 and y = 0,0023x + 0,0543, respectively.

## Discussion

The unprecedented spatial resolution here employed revealed a nearly perfect parapatric distribution of mtDNA lineages in the evenly distributed *Fucus ceranoides*, set by two dramatic, very narrow (<40 km) phylogeographic discontinuities (between CAM and ANL and between CED and ORT). There was a remarkable congruence between the nuclear (microsatellite) and the mitochondrial (mtIGS) data, at two levels. First, the multi-locus genotypes of *F. ceranoides* were broadly grouped (FCA and Structure analyses) in two main higher-level clusters, one of which was further subdivided,

resulting in 3 clusters, matching the mtIGS-based phylogroups. Second, the hierarchical clustering of genotypes was in agreement with the mtIGS-based phylogenetic inference of an earlier divergence of the phylogroup A (W sector) and the more recent split of phylogroups B and C (NW and N sectors respectively) from a common ancestral pool.

*Allopatric divergence and secondary contact*

The historical isolation, divergence and enduring integrity of three distinct and disjunct genetic pools at such a narrow spatial scale are as remarkable as puzzling. The congruence between the mtDNA matrilines and nuclear background shows that genetic differentiation in NW Iberian *F. ceranoides* is genome-wide, which excludes stochastic or selective sweeps as the drivers for the drastic mtDNA shifts. Also, the depth of genomic differentiation between phylogroups is high and characteristic of vicariant sub-populations that have long been diverging independently through accumulation of *de novo* mutations, drift and lineage sorting. Currently there are no large, estuarine-free regions along this shoreline and therefore distance *per se* cannot be invoked as a major contemporary factor accounting for the much deeper genomic differentiation among than within NW Iberian genetic sectors. The mean distance separating any two neighbouring populations (~32km) and neighbouring populations across phylogeographic breaks (<40km) are of the same order of magnitude, and several peripheral populations are geographically closer to populations across the phylogeographic breaks than they are to their nearest population within the same sector.

The parapatric divergence of Iberian *F. ceranoides* seems rather unlikely. The divergence between interior mtIGS haplotypes (*A1*, *B1* and *C1*; 4-5 mutations) is about one fifth that between *F. ceranoides* and *F. vesiculosus* (21-23 mutations), which are estimated to have diverged between 0.73 and 377 million years ago (Cánovas *et al. in*

*press*), estimate based on a calibrated phylogeny of 13 nuclear genes). Assuming a constant molecular clock, the divergence of phylogroups of *F. ceranoides* could date back to 165-867 ky BP. This indirect estimate is probably inaccurate, but implies that phylogroups start diverging well before the LGM. The high levels of genetic endemism and diversity of the Iberian region agree with the expectations for long-term persistence in glacial refugia. However, past climate and sea level changes have had global effects and have also affected Iberian refugial areas as well (Chao *et al.* 2002; Roucoux *et al.* 2005; Naughton *et al.* 2009). The periodic transgressions and regressions associated with the expansion/melting of the land-based ice-sheets caused continuous geographical rearrangement of near-shore habitats (Fauvelot *et al.* 2003; Graham *et al.* 2003), estuaries included. The geographic locations of NW Iberian estuaries in the past were different from today, as were to some extent the climatologic, oceanographic and hydrologic regimes (Roucoux *et al.* 2005; Naughton *et al.* 2009). Even discounting significant changes in the density and location of NW Iberian estuaries throughout past millennia, it appears rather improbable that any oceanographic feature acting as a powerful demographic filter could have remained relatively static in approximately its current (and very narrow) positions during such a long and dynamic period.

The modern genetic sectors in Iberian *F. ceranoides* are more likely to result from the regional reassembly of vicariant phylogroups into their current distributions following a period of independent, mostly allopatric divergence in past contracted areas of occurrence. This scenario implies the historical fragmentation and divergence of Iberian *F. ceranoides* in separate refugia (refugia within refugia), the subsequent expansion of these vicariant phylogroups along contiguous shorelines, and very limited gene-flow across meanwhile established secondary contact zones. The mismatch analyses and the distribution of the mtIGS variation are compatible with this scenario of

independent range expansions. Within each sector, the only widespread haplotypes are the interior (presumably ancestral) haplotypes, whereas derived haplotypes (presumably younger) are typically restricted to single populations. In the terrestrial realm, similar expansion/contraction cycles have been invoked to explain the mostly parapatric distribution of lineages and sister-species in a range of Iberian taxa currently displaying relatively continuous distributions (Gómez & Lunt 2007; Rodriguez-Sanchez *et al.* 2010; Feliner 2011).

The regular climatic changes and the dynamic shoreline/drainage geography across the Pleistocene glacial/interglacial cycles (Chao *et al.* 2002; Roucoux *et al.* 2005; Naughton *et al.* 2009) have probably played an important role in the regional range dynamics of Iberian *F. ceranoides,* but it is impossible with the present data to establish the specific drivers and its spatio-temporal contexts with detail. The chronology of the most recent expansions (leading to secondary contacts) are particularly elusive, but our data permit us to speculate on the temporal sequence of colonization of NW Iberia if haplotypic diversity within each sector is assumed to represent a good proxy for the time since colonization. The arrival of phylogroup C to northern Galicia likely post-dates the establishment of the genetically more diverse phylogroup A in Western Iberia, as there was clearly enough time for mtDNA diversification in the latter. Indeed, most populations of phylogroup A harbour private haplotypes in relatively high frequencies and some even local haplotype radiations. Remarkably, the number of haplotypes found in these 8 estuaries sampled along a coastline sector as small as 150 km far exceeds the number found in central and northern Europe [$N_{pop}$=12; (Neiva *et al.* 2010)]. The current distribution of this phylogroup probably represents a stable interglacial rear-edge that may have experienced southward expansions during colder periods such as the last glaciation. In contrast, the entire N sector is dominated by the ancestral haplotype

*C1*. A few derived *C1* haplotypes were found further East, in the populations of VIL, SAN and BAY, which suggests that phylogroup C arrived only later to NW Iberia from an eastern Cantabrian refugium. Finally, the 6 populations forming relict phylogroup B are probably close to their refugial distribution centred on the Artabro Gulf.

In this contraction/expansion scenario, the enduring integrity of the fine-scale phylogeographic structure within *F. ceranoides* can only be explained by very limited gene-flow across phylogeographic breaks. Migration may be particularly depressed there due to the presence of modern oceanographic barriers to dispersal, or be as low as elsewhere and simply reflect the inherent low vagility of the species.

*Evidence for oceanographic barriers to dispersal is lacking*

The most distinctive feature of NW Iberia coastline is its "rias", river valleys that were drowned with the marine transgression that followed the last glaciation. These rias are generally divided, based on their orientation, size and main geomorphological elements, into "Lower Rias" (between VIG and XAL), "Middle Rias" (between LIR and CED), and "Higher Rias" (eastwards of ORT), with the transitions at Cape Fisterra and Cape Ortegal, respectively. The modern distribution of mtDNA lineages of *F. ceranoides* matches remarkably well these subdivisions, but establishing a link between these geographic and genetic sub-divisions remains difficult, as their circumscriptions are not based on distinctive climatic, hydrological or oceanographical features that could be relevant in terms of the species ecology or dispersal.

The movement of buoyant, surface-drifting seaweed rafts is constrained by near-shore circulation patterns, mainly driven by winds, coastline morphology, tidal currents and river plumes, although the prevailing shelf/slope circulation patterns also play a role in offshore transport. It is impossible at the study scale to track the movements and fate

of reproductive drifters leaving/arriving estuaries and therefore to directly estimate the migration rates between populations within and across sectors. However, several lines of evidence do not suggest the existence of any specific seascape feature generating persistent (year-round) physical discontinuities matching the location of the observed genetic breaks. In NW Iberia, circulation patterns are complex and seasonally variable (Varela *et al.* 2005; Ruiz-Villarreal *et al.* 2006). During Autumn-Winter (downwelling season), SW winds prevail and a poleward current flows over the Western slope, with inter-annual variability in intensity and penetration into the Cantabrian Sea. During Spring-Summer (upwelling season), prevailing winds shift to become predominantly NE/N oriented, and an east/southward current develops over the shelf. These characteristic patterns are intermittently dominated by short-scale meteorological events that regionally intensify or reverse circulation during short periods in each season (Alvarez *et al.* 2009). The physical continuity of this coastline is well illustrated by the fate of the oil spilled by the Prestige tanker 250 km west off Cape Fisterra. The leaked (buoyant) fuel reached Cantabria (830 km from the sinking point) in just 17 days and spread along the Spanish shoreline from Vigo to the Basque Country (Castanedo *et al.* 2006), i.e., throughout and beyond the region here studied.

The available genetic evidence seems to confirm this. In the presence of barriers, genetic discontinuities would be expected to exist and be concordant, in location and eventually in depth, in co-occurring species sharing similar dispersal characteristics. However, most population genetic studies of shallow coastal biota focus on organisms with planktonic dispersive stages, and/or have very poor sampling resolution in the studied region. Demes from both the Western and Northern coasts of Galicia have been analyzed in mussels (Diz & Presa 2009), stalked barnacles (Quinteiro *et al.* 2007), spider and swimming crabs (Sotelo *et al.* 2008; Sotelo *et al.* 2009), flatfish (Bouza *et al.*

2002), octopus (Cabranes *et al.* 2008) and direct-developing cephalopods (Sanjuan *et al.* 1996). Their common denominator is the absence of genetic structure, indicating widespread connectivity by marine currents over NW Iberia. The single exception is the low dispersal, ovoviviparous snail *Littorina saxatilis*, which showed mild subdivision north and south of Cape Fisterra (Pineira *et al.* 2008). The authors propose that this break coincides with a putative ecological barrier but do not exclude the alternative hypothesis that it represents a secondary contact zone where allopatrically diverged sub-populations are being homogenized very slowly. The swift spread of the invasive seaweed *Sargassum muticum* throughout Galicia, Cantabria and Portugal, shortly after its first detection in Asturias (1980) and in Galicia (1986; (Incera *et al.* 2010)) also fails to support oceanographic barriers to drifting seaweed dispersal in this area.

The absence of prominent seascape or ecological barriers matching the phylogeographic breaks implies two things. First, that their positions may be rather contingent and simply reflect the idiosyncratic sequence of (re)colonization of NW Iberian estuaries by the three phylogroups during past range expansions; and second, that the unusually sharp genetic discontinuities at secondary contact zones are maintained despite normal migration rates.

*Colonization history vs. ongoing gene-flow*

A pattern of approximate stepping stone expansions originating in different refugia could promote the formation of genetic sectors even in the absence of dispersal barriers. This possibly reflects a relatively short viability of reproductive structures after frond dislodgement, or a density-dependent effect. *F. ceranoides* is dioecious and therefore effective estuarine colonization requires at least one male and one female fertile frond to be in close contact after dispersal (while synchronously releasing gametes) to produce *in*

*situ* the foundational zygotes that will eventually initiate a new population. Entangled mats of drifting viable male and female *F. ceranoides* are more likely to form near established populations that may export significant amounts of freshly dislodged drifters. Successful colonization across intermediate and larger distances surely occurs, as exemplified by the haplotype sharing of ANL and CED, or the colonization of Norway (across the North Sea) and Iceland, but it is probably much less frequent. Anyway, the process will self-reinforce: if populations in the interior of a sector go extinct, favoured recolonization from nearby sources will preserve the pre-existing genetic pattern.

Rare effective inter-estuarine dispersal, although allowing the colonization of unoccupied estuaries, leaves little importance for gene-flow in counteracting differentiation between fully established populations, slowing or preventing any progress towards migration-drift equilibrium. The remarkable genetic homogeneity of northern Europe, which was colonized post-glacially, clearly demonstrates the lack of gene-flow from the interior of the species range, where *F. ceranoides* exhibits considerably more diversity (Neiva *et al.* 2010; Neiva *et al. in press*). This study goes further in demonstrating that the effects of gene-flow are remarkably insignificant even at the shortest possible scale – between consecutive estuaries. Indeed, most populations in the W and NW sectors harbour private haplotypes in relatively high frequencies, including those located inside the same drainage systems. If migration is typically so low that even consecutive populations within sectors are genetically independent, it will also be unable to readily smooth pre-existing differentiation remaining from the colonization process.

*Density-barrier effects*

The primacy of colonization history over modern gene-flow reflects a poorly connected metapopulation system regulated by dispersal processes that are only effective within very restricted spatial and temporal windows. The apparent paradox of extremely limited population connectivity (here reported within and between sectors) despite an evident colonization potential (at least in the long-term) suggests extreme effects of population density in blocking gene flow. While the initial colonizers may expand the population relatively free of competition and have a disproportionate impact on the genetic make-up of establishing populations, once populations become fully established the large disparity in the number of residents (descending from the colonists) and subsequent immigrants act as a demographic buffer against changes in allele frequencies (Boileau *et al.* 1992; De Meester *et al.* 2002).

Organisms such as *F. ceranoides* that inhabit patchy habitats and possess the capacity for rapid population growth and habitat saturation (compared to immigration rates) are expected to be particularly prone to experiencing these effects. *Fucus spp.* are fecund, fertilization success is typically very high and recruitment in the vicinity of parental plants can be very efficient (Brawley 1992; Pearson & Serrao 2006). Monopolization of local space by marine *Fucus* species can be fast compared to their spread along unoccupied discontinuous shores (Arrontes 2002). Compared to its marine congeners, *F. ceranoides* may occupy vacant estuaries even faster and to a larger extent. The enclosed and sheltered nature of its habitat should improve the number and success of spawning events (Serrão *et al.* 1996b). Furthermore, *F. ceranoides* is a structural species that frequently forms monospecific (and often compact) belts within its particular tidal/salinity range. Contrary to the open shore where saturated communities compete for space, its estuarine habitat is free of similar competitors, potentially contributing for increased growth rates, densities and space monopolization.

The limited lineage admixture here reported indeed suggests that *F. ceranoides* has relatively short temporal windows of opportunity between estuarine colonization and saturation during which rare immigration can potentially result in detectable gene-flow. Incipient reproductive isolation (pre- or post-zygotic) can add to density effects and further depress gene-flow between divergent phylogroups (Tellier *et al.* 2011). The admixed nuclear background of LIR and CAM show that these divergent lineages of *F. ceranoides* can interbreed and should not be regarded as incipient species, although hybridization is common between *Fucus* species (Coyer *et al.* 2002a; Engel *et al.* 2005). Importantly, the shallow population structure within sectors shows that gene-flow is also reduced between more closely related populations. Unrecognized biophysical, ecological or reproductive barriers remain valid (not mutually exclusive) alternatives to pure demographic effects, but are probably not the most important factor underlying the apparent lack of gene-flow across sectors that maintains the parapatric structure of Iberian *F. ceranoides.*

In revealing that populations of *Fucus ceranoides* are to a large extent bounded by extinctions and (re)colonisations, our results challenge earlier beliefs that marine species are mainly unified by gene-flow. In this seaweed, connectivity estimates based on allele frequency divergence (e.g. FST and related measures) are inflated (within sectors) or depressed (across secondary contact zones) to a great extent by historical colonization processes. Despite its evident parapatric structure, an IBD correlation was unexpectedly recovered in NW Iberian *F. ceranoides*, showing that IBD patterns can arise where a causal relationship between distance and gene-flow is missing. Such spurious correlations have been noted in other highly structured species (Ramachandran *et al.* 2005; Mills *et al.* 2007), and confirm that gene-flow may not be the decisive factor underlying many significant associations between geographic and genetic distances.

*Conclusions*

Our fine-scale, multi-marker approach revealed sharply disconnected population units in NW Iberian *Fucus ceranoides*. The levels of differentiation and the absence of modern habitat discontinuities or prominent ecological/oceanographic barriers to dispersal indicate that its remarkable genetic structure is the product of past range dynamics (including contractions, sequential expansions and secondary contact) coupled with very strong density-barrier effects. These conclusions are highly relevant to other organisms with rare and spatially restricted dispersal, helping explain the apparent paradox of extensive genetic subdivision in geographically restricted refugial regions (indicating very limited connectivity) despite obvious colonization abilities of these same species at larger spatio-temporal scales [e.g. allowing extensive post-glacial range expansions, see also (Fraser *et al.* 2009b)]. These species may not fit the conventional "low-dispersal" or "high-dispersal" dichotomy, since rare dispersal into vacant (colonization) and saturated (immigration) habitats can have fundamentally different demographic and genetic effects.

This study also supports the view that the patterns of genetic structure and differentiation in marine-restricted dispersers often reflect persistent non-equilibrium conditions (Pelc *et al.* 2009). In particular, it shows that distant (but rather similar) populations do not necessarily exchange more migrants than closer (but very divergent) populations, and that steep genetic breaks are not necessarily maintained by extrinsic dispersal barriers. The regular climatic oscillations and the transitory nature of near-shore habitats may actually prevent low dispersal marine species in general from ever attaining migration-drift equilibrium at most spatial scales. Inferring patterns of connectivity from genetic data alone may thus be misleading, since historical patterns of

extinction and colonization will be more important than ongoing gene-flow in determining the extent of genetic differentiation between modern populations.

**Acknowledgements**

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## Supplementary material

## Figures

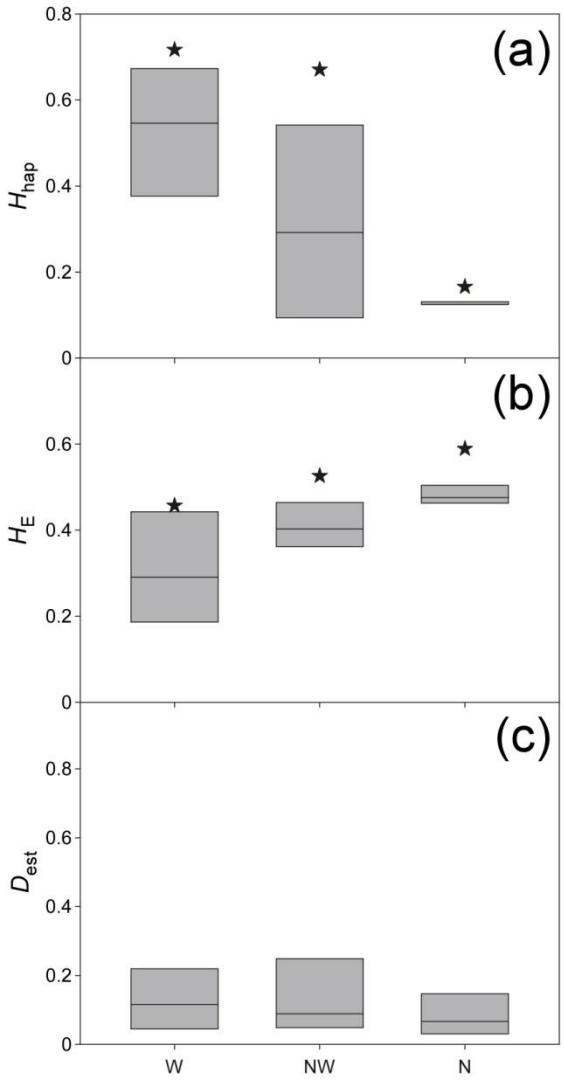


**Figure 4.S1** Genetic diversity and differentiation of populations of *Fucus ceranoides* within Western (W), Northwestern (NW) and Northern (N) sectors. **(a)** Haplotype diversity ($H_{hap}$) at population (box-plots) and sector (stars) levels. **(b)** Nei's gene diversity ($H_E$) at population (box-plots) and sector (stars) levels. **(c)** Box-plot of pairwise differentiation of populations ($D_{est}$) within regions. Box-plots depict the median (horizontal line) and the 25$^{th}$ and 75$^{th}$ percentiles (bottom and top of the box).

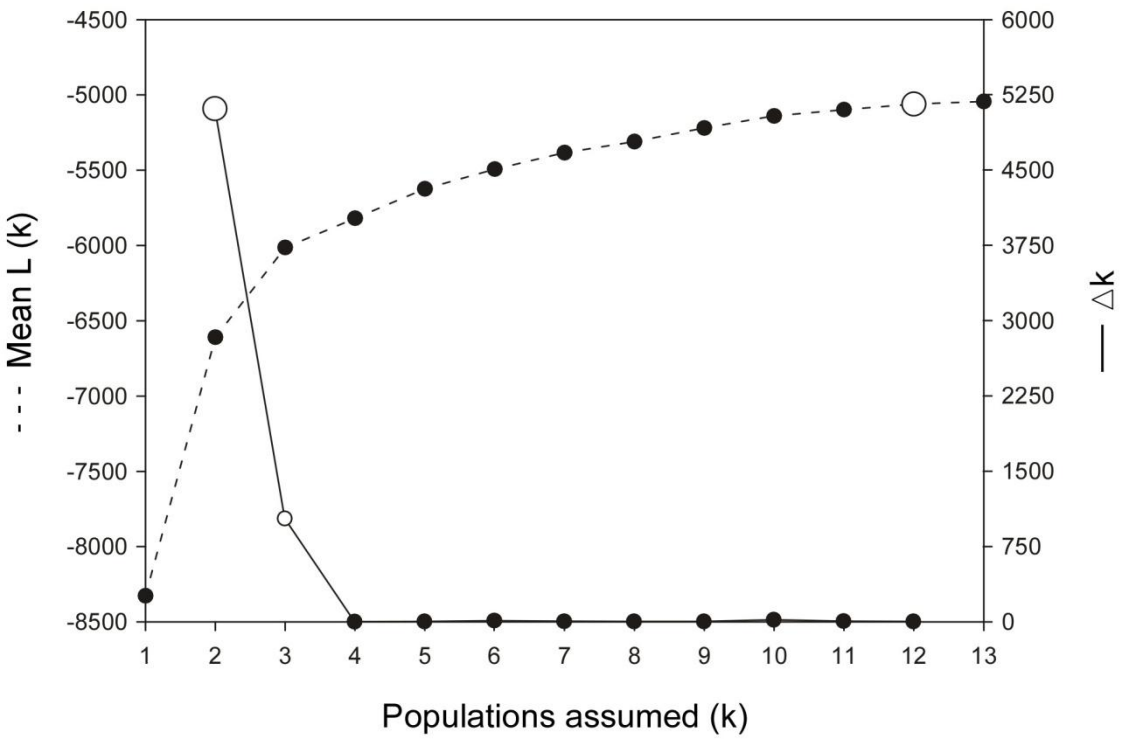


**Figure 4.S2** Most probable number of genetic clusters of Iberian *Fucus ceranoides* according to STRUCTURE. Five iterations were run for each number of genetic clusters assumed (*K*). The most probable *K* (open symbols) were inferred with Pritchard *et al.* (2000; left axis) and Evanno *et al.* (2005; right axis) choice criteria.

## Tables

**Table 4.S1** – Estimates of pairwise differentiation between the 26 Iberian populations of *Fucus ceranoides*. *F*ST (θ) values are given above diagonal and Jost's *D*est below diagonal. Non-significant *F*ST values (1000 permutations) are depicted in bold. Population codes are given in Table 4.1.

| | **VIA** | **VIG** | **PON** | **UMI** | **ULL** | **NOI** | **XAL** | **LIR** | **CAM** | **ANL** | **RCO** | **BET** | **ARE** | **FER** | **CED** | **ORT** | **BAR** | **VIV** | **FAZ** | **FOZ** | **VEG** | **POR** | **NAV** | **VIL** | **SAN** | **BAY** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VIA** | --- | 0.143 | 0.094 | 0.223 | 0.437 | 0.381 | 0.279 | 0.579 | 0.534 | 0.666 | 0.649 | 0.677 | 0.659 | 0.654 | 0.702 | 0.643 | 0.571 | 0.625 | 0.621 | 0.609 | 0.635 | 0.628 | 0.628 | 0.639 | 0.642 | 0.599 |
| **VIG** | 0.011 | --- | **0.031** | **0.021** | 0.138 | 0.312 | 0.222 | 0.499 | 0.423 | 0.617 | 0.582 | 0.618 | 0.601 | 0.601 | 0.655 | 0.598 | 0.547 | 0.591 | 0.590 | 0.592 | 0.624 | 0.605 | 0.585 | 0.607 | 0.626 | 0.576 |
| **PON** | 0.006 | <0.001 | --- | 0.076 | 0.232 | 0.340 | 0.244 | 0.550 | 0.480 | 0.648 | 0.625 | 0.657 | 0.639 | 0.636 | 0.685 | 0.626 | 0.568 | 0.615 | 0.613 | 0.612 | 0.644 | 0.629 | 0.617 | 0.631 | 0.651 | 0.601 |
| **UMI** | 0.010 | 0.001 | 0.005 | --- | **0.033** | 0.332 | 0.195 | 0.468 | 0.394 | 0.594 | 0.552 | 0.600 | 0.584 | 0.582 | 0.634 | 0.580 | 0.538 | 0.579 | 0.580 | 0.593 | 0.629 | 0.601 | 0.573 | 0.597 | 0.630 | 0.575 |
| **ULL** | 0.022 | 0.007 | 0.011 | 0.001 | --- | 0.416 | 0.269 | 0.506 | 0.418 | 0.628 | 0.597 | 0.644 | 0.624 | 0.617 | 0.677 | 0.623 | 0.589 | 0.626 | 0.625 | 0.643 | 0.683 | 0.650 | 0.613 | 0.643 | 0.683 | 0.627 |
| **NOI** | 0.071 | 0.075 | 0.075 | 0.078 | 0.097 | --- | 0.297 | 0.516 | 0.369 | 0.526 | 0.588 | 0.550 | 0.539 | 0.534 | 0.551 | 0.503 | 0.468 | 0.508 | 0.496 | 0.512 | 0.528 | 0.505 | 0.505 | 0.511 | 0.541 | 0.518 |
| **XAL** | 0.081 | 0.057 | 0.091 | 0.041 | 0.067 | 0.172 | --- | 0.291 | 0.195 | 0.414 | 0.333 | 0.388 | 0.381 | 0.381 | 0.450 | 0.377 | 0.340 | 0.377 | 0.375 | 0.398 | 0.419 | 0.399 | 0.350 | 0.393 | 0.436 | 0.381 |
| **LIR** | 0.424 | 0.368 | 0.373 | 0.297 | 0.262 | 0.596 | 0.189 | --- | 0.325 | 0.337 | 0.235 | 0.324 | 0.358 | 0.292 | 0.404 | 0.342 | 0.333 | 0.396 | 0.387 | 0.420 | 0.486 | 0.412 | 0.359 | 0.439 | 0.500 | 0.409 |
| **CAM** | 0.234 | 0.220 | 0.218 | 0.157 | 0.145 | 0.160 | 0.134 | 0.254 | --- | 0.388 | 0.431 | 0.414 | 0.393 | 0.356 | 0.393 | 0.290 | 0.304 | 0.312 | 0.299 | 0.387 | 0.391 | 0.347 | 0.298 | 0.326 | 0.473 | 0.416 |
| **ANL** | 0.937 | 0.844 | 0.879 | 0.730 | 0.673 | 0.690 | 0.607 | 0.337 | 0.516 | --- | 0.284 | 0.181 | 0.150 | 0.096 | 0.197 | 0.284 | 0.289 | 0.336 | 0.334 | 0.357 | 0.454 | 0.347 | 0.313 | 0.384 | 0.365 | 0.298 |
| **RCO** | 0.478 | 0.407 | 0.430 | 0.330 | 0.298 | 0.639 | 0.281 | 0.119 | 0.383 | 0.104 | --- | 0.226 | 0.336 | 0.279 | 0.409 | 0.369 | 0.363 | 0.442 | 0.448 | 0.472 | 0.543 | 0.457 | 0.383 | 0.505 | 0.482 | 0.430 |
| **BET** | 0.568 | 0.492 | 0.523 | 0.399 | 0.371 | 0.495 | 0.329 | 0.205 | 0.286 | 0.072 | 0.048 | --- | 0.167 | 0.136 | 0.360 | 0.318 | 0.339 | 0.427 | 0.430 | 0.456 | 0.532 | 0.434 | 0.387 | 0.482 | 0.458 | 0.365 |
| **ARE** | 0.593 | 0.507 | 0.535 | 0.416 | 0.384 | 0.536 | 0.344 | 0.301 | 0.294 | 0.076 | 0.089 | 0.024 | --- | 0.055 | 0.358 | 0.365 | 0.339 | 0.345 | 0.378 | 0.393 | 0.486 | 0.401 | 0.346 | 0.410 | 0.349 | 0.237 |
| **FER** | 0.753 | 0.655 | 0.686 | 0.557 | 0.513 | 0.646 | 0.447 | 0.227 | 0.313 | 0.048 | 0.098 | 0.064 | 0.029 | --- | 0.307 | 0.320 | 0.305 | 0.330 | 0.339 | 0.358 | 0.442 | 0.341 | 0.294 | 0.381 | 0.356 | 0.268 |
| **CED** | 0.913 | 0.814 | 0.846 | 0.706 | 0.658 | 0.556 | 0.498 | 0.307 | 0.315 | 0.112 | 0.279 | 0.249 | 0.304 | 0.250 | --- | 0.293 | 0.289 | 0.314 | 0.313 | 0.395 | 0.450 | 0.386 | 0.354 | 0.411 | 0.503 | 0.446 |
| **ORT** | 0.764 | 0.657 | 0.675 | 0.553 | 0.541 | 0.522 | 0.419 | 0.289 | 0.209 | 0.240 | 0.166 | 0.197 | 0.277 | 0.292 | 0.198 | --- | 0.031 | 0.122 | 0.122 | 0.282 | 0.293 | 0.165 | 0.185 | 0.233 | 0.368 | 0.295 |
| **BAR** | 0.698 | 0.599 | 0.594 | 0.594 | 0.672 | 0.602 | 0.434 | 0.382 | 0.331 | 0.315 | 0.240 | 0.323 | 0.305 | 0.288 | 0.289 | 0.008 | --- | 0.053 | 0.070 | 0.178 | 0.181 | 0.114 | 0.161 | 0.201 | 0.280 | 0.233 |
| **VIV** | 0.730 | 0.605 | 0.618 | 0.548 | 0.587 | 0.529 | 0.421 | 0.464 | 0.244 | 0.318 | 0.274 | 0.322 | 0.197 | 0.264 | 0.219 | 0.013 | 0.005 | --- | 0.049 | 0.214 | 0.196 | 0.143 | 0.173 | 0.160 | 0.294 | 0.261 |
| **FAZ** | 0.742 | 0.617 | 0.630 | 0.573 | 0.619 | 0.533 | 0.439 | 0.464 | 0.251 | 0.335 | 0.384 | 0.413 | 0.246 | 0.247 | 0.266 | 0.062 | 0.054 | 0.018 | --- | 0.102 | 0.082 | 0.047 | 0.085 | 0.148 | 0.346 | 0.295 |
| **FOZ** | 0.702 | 0.679 | 0.656 | 0.743 | 0.841 | 0.670 | 0.495 | 0.577 | 0.382 | 0.425 | 0.643 | 0.542 | 0.464 | 0.323 | 0.352 | 0.283 | 0.152 | 0.178 | 0.082 | --- | 0.071 | 0.153 | 0.178 | 0.197 | 0.345 | 0.286 |
| **VEG** | 0.564 | 0.594 | 0.550 | 0.662 | 0.748 | 0.513 | 0.486 | 0.587 | 0.271 | 0.605 | 0.623 | 0.638 | 0.445 | 0.374 | 0.424 | 0.247 | 0.188 | 0.151 | 0.029 | 0.026 | --- | 0.099 | 0.160 | 0.216 | 0.374 | 0.357 |
| **POR** | 0.637 | 0.551 | 0.539 | 0.568 | 0.649 | 0.490 | 0.390 | 0.383 | 0.268 | 0.407 | 0.393 | 0.450 | 0.334 | 0.304 | 0.272 | 0.101 | 0.058 | 0.072 | 0.014 | 0.095 | 0.053 | --- | 0.077 | 0.198 | 0.291 | 0.279 |
| **NAV** | 0.669 | 0.561 | 0.586 | 0.486 | 0.481 | 0.493 | 0.295 | 0.268 | 0.178 | 0.345 | 0.288 | 0.308 | 0.209 | 0.189 | 0.231 | 0.133 | 0.168 | 0.134 | 0.036 | 0.080 | 0.047 | 0.035 | --- | 0.233 | 0.317 | 0.295 |
| **VIL** | 0.756 | 0.638 | 0.666 | 0.593 | 0.639 | 0.578 | 0.437 | 0.595 | 0.291 | 0.464 | 0.559 | 0.509 | 0.400 | 0.385 | 0.356 | 0.120 | 0.164 | 0.074 | 0.059 | 0.074 | 0.082 | 0.095 | 0.106 | --- | 0.299 | 0.237 |
| **SAN** | 0.555 | 0.562 | 0.527 | 0.622 | 0.711 | 0.466 | 0.510 | 0.607 | 0.494 | 0.312 | 0.383 | 0.330 | 0.226 | 0.253 | 0.484 | 0.248 | 0.237 | 0.138 | 0.204 | 0.228 | 0.200 | 0.132 | 0.201 | 0.136 | --- | 0.136 |
| **BAY** | 0.568 | 0.509 | 0.499 | 0.537 | 0.625 | 0.626 | 0.362 | 0.444 | 0.494 | 0.338 | 0.385 | 0.339 | 0.151 | 0.185 | 0.515 | 0.271 | 0.203 | 0.225 | 0.205 | 0.210 | 0.215 | 0.115 | 0.139 | 0.139 | 0.049 | --- |

# CHAPTER V. GENERAL CONCLUSIONS

The main conclusions of this thesis are summarized below in separate sections, each corresponding to a specific topic.

## *Fucus* phylogeny

This study helped clarify some phylogenetic relationships within *Fucus*. The combination of nuclear, mitochondrial and chloroplast sequence data demonstrated the clear demarcation of *F. ceranoides* from the *F. vesiculosus / F. spiralis* complex. Indeed, divergence levels in all surveyed loci indicated a relatively old split between the two clades, contradicting early suggestions of a closer relationship of this estuarine species with its marine congeners (Billard et al., 2005; Coyer et al., 2006). Indirectly, this study suggests that dioecy (and not monoecy) is the ancestral mating system in this group.

## Gene surfing

Phylogenetic analyses also allowed the detection of an asymmetric introgression of a single *F. vesiculosus* cytoplasm into *F. ceranoides*, which was not accompanied by introgression at the nuclear background. The historical causes underlying this cyto/nuclear conflict are hard to interpret in the absence of modern hybrid zones, but geneomic incongruences are nevertheless commonplace in other taxonomic groups. Organelle capture was inferred to have occurred rather recently and its extensive distribution inferred to be associated with the post-glacial range expansion of *F. ceranoides*. Although previous studies have reported the role of genetic surfing in producing extensive introgression, *F. ceranoides* represents the first example of such

phenomena in a marine organism. More generally, these data highlight the importance of an adequate genomic and taxonomic sampling when introgression between related species is suspected.

**Historical biogeography**

The palaeoclimatic oscillations in the N Atlantic have driven important biogeographical shifts in *F. ceranoides* and played a significant role in shaping its genetic make-up. The seaweed revealed a clear poleward decrease in allelic richness, gene diversity, mtDNA diversity and within-region population differentiation, which supported the long-term refugial status of Iberia and the post-glacial colonization of previously glaciated/emersed northern latitudes. The persistence of *F. ceranoides* at periglacial latitudes during the last glaciation remained ambiguous. Remarkable differences in range-dynamics were apparent between *F. ceranoides* and some of its marine relatives (e.g. *F. serratus* and *Ascophyllum nodosum*), in particular the long-term persistence and/or stability of populations at southernmost (NW Iberia, present interglacial) and northernmost (Celtic Sea/English Channel, full glacial) rear edges. These differences highlight the individualistic nature of species responses to climatic change.

**Rafting, population's connectivity and habitat tracking**

*Fucus ceranoides*, as expected from its life-cycle and estuarine habitat, lies in the low end of the marine dispersal continuum. Sharp genetic discontinuities (e.g. fixed haplotypic/allelic differences), both shallow and deep, are pervasive throughout its range, and indicate that the historical (colonization/founder events) and recurrent (isolation/drift) processes contributing to population differentiation are weakly counteracted by gene-flow. Apparently, migration rates allowed by rafting are

intrinsically so low that even sequential populations are to a large extent genetically independent. The sharp phylogeographic breaks found in NW Iberia (between lineages A, B and C) and Brittany/English Channel (Lineages C and I) highlight the irrelevance of recurrent gene flow in smoothing pre-existing historical differences between highly divergent but spatially close populations even in the absence of extrinsic dispersal barriers. As a consequence, the modern population structure of *F. ceranoides* largely reflects historical, non-equilibrium conditions resulting from patterns of estuarine colonization and strong density-barrier effects, rather than modern patterns of gene flow.

*F. ceranoides* provides a remarkable example of how infrequent and spatially limited dispersal can have contrasting effects at the scales of meta-population (connectivity) *versus* range dynamics (habitat tracking), and how dispersal restrictions can result in either genetic divergence (refugial areas) or homogeneity (recently colonized areas) depending on the maturity (age) and demographic conditions of the populations. Despite the limited importance of rafting in mediating gene-flow among populations of *F. ceranoides*, the extensive northward expansion of the species following the LGM show that dispersal of drifting fertile fronds still allows the successful colonization of unoccupied shores in the long term. This shows that colonization (successful dispersal into vacant habitats) and immigration (successful dispersal into established populations), as sources of gene-flow, have very different genetic effects. This is because gene flow depends not only on the number of migrants arriving, but also on the demographic properties of the receiving populations.

More generally, this study shows that inferring patterns of connectivity from genetic data alone may be misleading, especially in low-dispersal species depending on highly discontinuous habitats and/or subjected to regular processes of extinction and

colonization. In these situations, non-equilibrium conditions can be pervasive and long-lasting. In addition, if suh organisms form dense local populations, density-barrier effects buffer the effects of rare migration, resulting in historical processes being more important than ongoing patterns of gene flow in determining the extent of genetic differentiation of modern populations. These findings are highly relevant to understand the population structure of low-dispersal marine species, many of which also display highly structured and hierarchical phylogeographic patterns in the absence of obvious dispersal barriers.

**Conservation**

NW Iberia was identified as a repository of unique genetic diversity of *F. ceranoides*. This region harbours a significant proportion of private genetic variation, including two endemic mtDNA lineages. The uniqueness of the region results from the long-term persistence and isolation of distinct refugial *F. ceranoides* populations there, but also from the fact that most of these relict populations did not participate in the post-glacial colonization. This geographically restricted region represents the modern rear edge of the species, and its loss as a result of ongoing climatic change would have a major effect on the overall genetic diversity of the species. The ubiquity of *F. ceranoides* throughout NW Iberia suggests this area still represents a stable rear-edge that has not turned into a marginal habitat during the course of the present interglacial. However, lineage B is represented by as few as 6 populations, and lineage A (assuming that all populations between Mondego and Camariñas belong to this lineage, which was not fully evaluated) by a maximum of 13 populations, making their extinction in the future a reasonable concern.

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