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Original Article

Hierarchical genetic structuring in the cool boreal kelp, *Laminaria digitata*: implications for conservation and management

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Kelp are foundation species threatened by ongoing warming trends and increased harvesting pressure. This emphasizes the need to study genetic structure over various spatial scales to resolve demographic and genetic processes underpinning resilience. Here, we investigate the genetic diversity in the kelp, *Laminaria digitata*, in previously understudied southern (trailing-edge) and northern (range-centre) regions in the Northeastern Atlantic Ocean. There was strong hierarchical spatial structuring with significantly lower genetic variability and gene flow among southern populations. As these span the area of the Hurd's deep Pleistocene glacial refuge, the current low variation likely reflects a fraction of previous levels that has been eroded at the species southern edge. Northern variability and private alleles also indicate contributions from cryptic northern glacial refugia. Contrary to expectations of a positive relationship between neutral genetic diversity and resilience, a previous study reported individuals from the same genetically impoverished southern populations to be better adapted to cope with thermal stress than northern individuals. This not only demonstrates that neutral genetic diversity may be a poor indicator of resilience to environmental stress but also confirms that extirpation of southern populations will result in the loss of evolved, not just potential, adaptations for resilience.

Keywords: genetic diversity, glacial refugia, range contraction, range centre, trailing edge

Introduction

Kelp are foundation species that form extensive forests along rocky coastlines in temperate and Arctic regions (Steneck et al., 2002; Smale et al., 2013; Teagle et al., 2017). These forests rank among the world's most diverse and productive ecosystems (Steneck et al., 2002; Smale et al., 2013) and deliver a range of ecosystem goods and services to human society (e.g. commercial fisheries, biogenic coastal defence) (Beaumont et al., 2008). Moreover, their role in coastal nutrient cycling, for example as "Blue Carbon" donors, is increasingly gaining attention

(Krause-Jensen and Duarte, 2016). Kelp distributions are strongly constrained by temperature (Eggert, 2012), making them sensitive to ongoing warming trends (Smale, 2020). Superimposed on broad-scale climatic changes is a range of other local human impacts (e.g. direct exploitation and eutrophication) that can also drive population loss (Krumhansl et al., 2016). Given the fundamental role that kelp play in underpinning the wider ecosystem, understanding the demographic processes and mechanisms of ecological resilience to ongoing threats will be fundamental for management and conservation.

Resolution of intraspecific genetic structure is a powerful way to understand the responses of species and populations to historical, current, and future environmental changes. Genetic studies of kelp populations have provided insights into the roles of life history, geographical distance, and other seascape factors in shaping contemporary connectivity patterns (Durrant et al., 2014; Durrant et al., 2018), which may influence local adaptation and resilience in various ways (Heino et al., 1997; Almany et al., 2009). Studies over wider geographical areas have also revealed pronounced signatures of historical contractions and expansion events associated with the Pleistocene glaciations (2.6 Ma to 12 ka) (Pielou, 1991; Webb and Bartlein, 1992; Hewitt, 1999). These cycles have resulted in former glacial refugial areas, typically at low-latitude range edges, often accumulating high levels of endemic genetic diversity, with decreasing diversity towards leading edges of postglacial colonization routes (Hewitt, 2004). This means that the bulk of species' genetic diversity often lies at its trailing edge, where populations are generally under the greatest threat from contemporary warming (Diez et al., 2012; Nicastro et al., 2013; Wernberg et al., 2013). The loss of endemic or unique genetic lineages represents a species level loss of evolutionary potential and, over shorter timescales, can reduce the physiological versatility and ecological resilience of kelp forest populations (Wernberg et al., 2018). Clearly, there is a pressing need to study genetic variation and its drivers, across broad spatial scales and within both trailing-edge and range-centre populations, to inform the conservation and management of kelp populations and the ecosystems they underpin (Almany et al., 2009; Coleman et al., 2017).

Laminaria digitata is a boreal kelp with a transatlantic distribution. In the Northeastern (NE) Atlantic, it is distributed from the Arctic to the English Channel where it is threatened by ongoing and predicted warming trends (Raybaud et al., 2013; Assis et al., 2018a; Hereward et al., 2020). As the English Channel has been identified as an important glacial refuge for several macroalgal species (Provan et al., 2005; Provan and Maggs, 2012), trailing-edge population loss may have serious genetic consequences for the wider gene pool of *L. digitata* (Assis et al., 2018a). To date, most genetic studies of *L. digitata* have been limited to French trailing-edge populations (but see Brennan et al., 2014) where significant structuring (Billot et al., 2003; Robuchon et al., 2014) compatible with its limited dispersal potential (Valero et al., 2011) has been reported. However, there have been no attempts to resolve the patterns of genetic diversity over broader spatial scales that encompass northern populations. This means that the relative importance of loss of southern populations for overall genetic diversity of *L. digitata* is still unknown. Here, we examined and compared genetic variation from northern and southern regions representing *L. digitata*'s contemporary range centre and trailing edge in the NE Atlantic. We employed a similar hierarchical sampling strategy to Robuchon et al. (2014) with overlapping marker sets and integrated data from both studies, which allowed us to explicitly compare southern populations in both the UK and French waters. As some of the sampled populations were also used in a previous common garden thermal stress response experiment (King et al., 2019), we were able to directly explore the relationship between neutral genetic variation, population demographics, and empirical resilience characteristics.

Material and methods

Study design

Sampling followed a hierarchical design based on a total of four established study regions in the United Kingdom (Smale et al., 2016; Figure 1). These consisted of two northern (N1 and N2) and two southern (S1 and S2) regions, representing *L. digitata*'s contemporary core range centre (N1 and N2) and peripheral trailing edge (S1 and S2). Within each of these four regions three sites were selected at least 10 km from one another (Figure 1). A sample was also collected from a single geographical outlier site in Norway. At each site, 30 individuals were haphazardly sampled from intertidal rocky reef habitats during periods of low tide emersion. Mature, healthy-looking, sporophytes located at least 5 m apart from one another were sampled by excising fresh tissue from directly above the meristem. Samples were dried in individual 1.5-ml tubes with silica drying crystals until DNA extraction.

DNA extraction and PCR conditions

Genomic DNA was isolated from 5 to 10 mg of dried tissue and ground to a fine powder using a ball mill. Five hundred microlitres of extraction buffer (100 mM Tris, 25 mM EDTA, 1.4 M NaCl, 1% PVP, and 2% CTAB, pH 8) was added, vortexed, and left at room temperature (RT) for 10 min. RNA was digested by adding 2 μl of RNAse A (10 mg/ml) to the solution and incubating at 55°C for 1 hr. Total DNA was extracted by chloroform extraction, 500 μl of chloroform:isoamyl alcohol (24:1, v/v) was added, vortexed vigorously, and centrifuged at 14 000*g* for 10 min, and the upper aqueous layer was transferred to a new tube. DNA was precipitated out with 30 μl of ammonium acetate and 200 μl of isopropanol at −20°C for 30 min. Samples were centrifuged at 14 000*g* for 10 min at RT. Two EtoH washes were performed at 70 and 95%, and pellets were recollected at 14 000*g* for 10 min at RT. Pellets were air dried for 1 hr and then resuspended in 50 μl of DEPC-treated water. Twelve microsatellite markers previously developed for *L. digitata* (Ld148, Ld158, Ld167, Ld371, Ld531, and Ld704) and *Laminaria ochroleuca* (Lo4-24, Lo454-17, Lo454-23, Lo454-24, Lo454-27, and Lo454-28) were used (Robuchon et al., 2014). Microsatellites were amplified by individual PCR in 10 μl of final volumes containing 1× GoTaq Flexi colourless reaction buffer, 2 mM $MgCl_2$, 150 μm dNTPs, 0.35 U GoTaq DNA polymerase (Promega), and 2 μl template (1:50 dilution) following the protocol of Robuchon et al. (2014). Amplicon fragment size was analysed on an ABI PRISM 377 automated DNA sequencer (Applied Biosystems), and alleles were scored manually using PEAKSCANNER 1.0.

Statistical analysis of microsatellite data

Measuring genetic structure

Genotype frequency conformance to Hardy–Weinberg expectations (HWE) and genotypic linkage equilibrium between pairs of loci were tested using exact tests (10 000 batches, 5000 iterations) in GENEPOP 3.3 (Rousset, 2008).

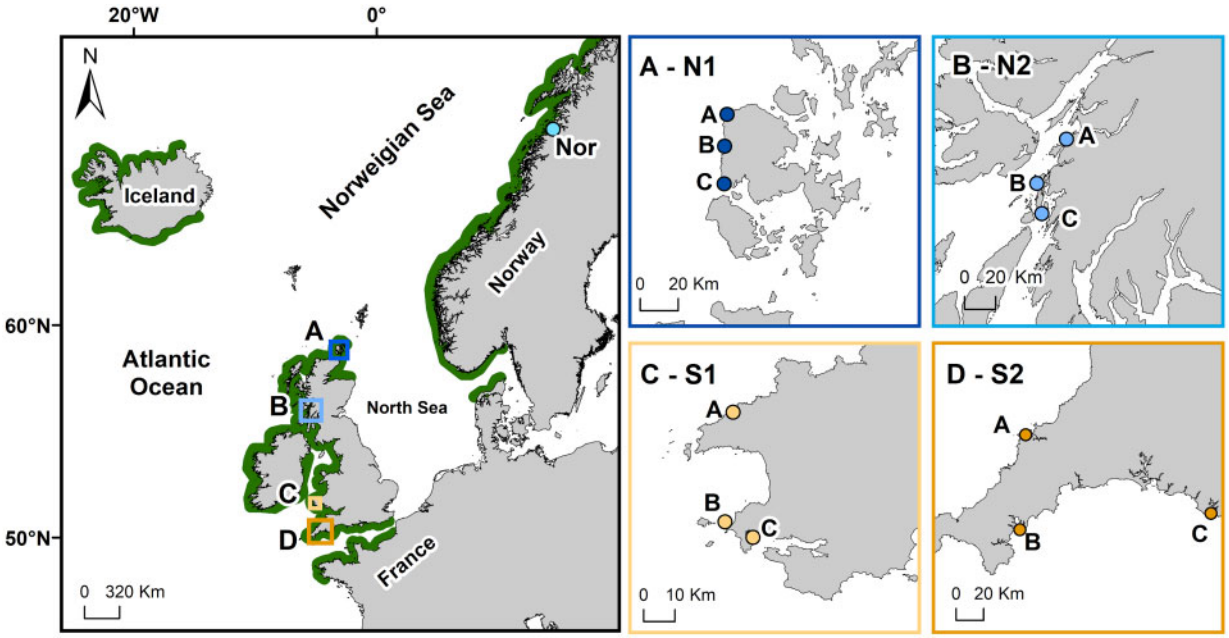

Figure 1. Sampling sites for *Laminaria digitata*. Extent rectangles represent corresponding study region.

Genetic structure was investigated using the Bayesian clustering method implemented in the programme STRUCTURE (Pritchard et al., 2000) to identify the most probable number of genetic clusters (K) (from a range of 1–13) within the data. The analysis was performed both with and without prior sample information (as recommended by Hubisz et al., 2009) and with multiple parameter permutations (admixture and correlated allele frequencies, as recommended by Pritchard et al., 2000). Each run consisted of a burn-in of 10^6 steps followed by 5×10^6 steps with three runs performed for each K model tested. Optimal models were assessed using ΔK (Evanno et al., 2005) and visual inspection of STRUCTURE bar plots. Genetic differentiation among samples was quantified using global and pairwise F_{ST} values with significance assessed with P values following 10 000 permutations in FSTAT (Goudet, 1995). F_{ST} values were also estimated using the null allele correction method in FreeNA (Chapuis and Estoup, 2007). Pairwise F_{ST} matrices were visualized using the principal coordinate analysis in GENALEX 6.2 (Peakall and Smouse, 2006). Mantel tests, implemented in GENALEX, were used to test for isolation by distance using the correlation between pairwise F_{ST} and geographical (shortest sea distances) distances between sample sites. Geographical distances were estimated by direct shipping distance between sites using NETPAS DISTANCCE 2.0 (Seafuture Inc.). Hierarchical Analysis of Molecular Variance (AMOVA; Excoffier et al., 1992) was performed in ARLEQUIN (Excoffier and Lischer, 2010) to partition genetic variance among groups of samples (F_{CT}) and among samples within groups (F_{SC}) with significances determined with 1000 permutations.

Measuring genetic variation

Genetic variation within samples was characterized using the number of alleles (N_a), allelic richness (A_r), observed heterozygosity (H_O), and expected heterozygosity (H_E), all calculated using GENALEX. Following Assis et al. (2018b), the number of private alleles was used as a measure of endemism and calculated in GENALEX. Randomization procedures in FSTAT were used to detect significant differences in heterozygosity, A_r, F_{IS}, F_{ST}, and relatedness (r) among user-defined groups of samples following 10 000 permutations. For comparisons integrating data from Robuchon et al. (2014), locus Lo454-27, not genotyped in that study, was omitted.

Results

Genetic structure

The total number of alleles per locus ranged from 4 (lo454-27) to 27 (LD-167) with an average of 13. Global tests revealed no significant linkage disequilibrium between any pair of loci except Lo454-17 and Lo454-28 ($P = 0.0003$). However, inspection of individual samples revealed significant disequilibrium tests' results between this locus pair at only three sites (N1-B, N2-C, and S1-A). Exclusion of any one of these samples resulted in a non-significant global disequilibrium test result. All loci were therefore considered independent and retained in downstream analyses.

Locus ($n = 12$) × sample ($n = 13$) tests of conformance to Hardy–Weinberg expectations revealed significant deviations in 53 of 156 tests, in all cases due to heterozygote deficits. These deviations were not associated with particular site–locus combinations. Excluding S2-A, which reported significant heterozygote deficit at only one locus, each site exhibited heterozygote deficits at an average of 4.5 loci, while each locus reported deficits at an average of 4.4 sites. Accordingly, all but two sites displayed highly significant positive F_{IS} values (Table 1).

Bayesian clustering analysis in STRUCTURE revealed a nearly identical pattern of hierarchical differentiation irrespective of the parameters employed. At $K = 3$, the best supported model indicated that the Norwegian sample was differentiated from the Northern samples (i.e. 3 groups = Norway; N1 + N2; and S1 + S2) (Figure 2). Subdivision at $K = 4$ differentiated N1 and N2 regions, while at $K = 5$, S1 and S2 were clearly partitioned. Analysis of larger K values showed no additional groupings.

F_{ST}-based analyses supported these results and provided further resolution by revealing significant differentiation among sites within

Table 1. Summary information for individual sites including sample sizes (N), mean allele number (N_a), allelic richness (A_r), observed and expected heterozygosities (H_O and H_E, respectively), and multilocus F_{IS} values.

Region and private alleles (P_a)	Site	Code	N	N_a	A_r	H_O	H_E	F_{IS}
Norway ($P_a = 13$)	Ness	Nor	30	4.50	3.33	0.39	0.49	0.205*
UK N 1 ($P_a = 20$)	Birsay	A	23	6.33	4.50	0.47	0.66	0.291*
	Bay of Skail	B	25	6.17	4.57	0.64	0.69	0.065*
	Warbeth	C	17	4.83	4.07	0.50	0.64	0.227*
UK N 2 ($P_a = 16$)	Ganovan	A	24	5.25	3.97	0.47	0.54	0.246*
	Easdale	B	25	6.25	4.37	0.52	0.61	0.154*
	Luing	C	17	5	4.00	0.37	0.49	0.316*
UK S 1 ($P_a = 8$)	Abereiddy	A	29	4.42	3.13	0.43	0.50	0.136*
	Martin's Haven	B	29	3.67	2.75	0.39	0.44	0.116*
	Dale	C	20	3.83	3.08	0.44	0.48	0.084
UK S 2 ($P_a = 17$)	Trevone	A	27	3.08	2.34	0.31	0.38	0.169*
	Saint Mawes	B	17	3.92	3.23	0.42	0.49	0.168*
	Plymouth	C	30	4.25	2.83	0.39	0.42	0.017

Reported are regional grouping of sites and the total number of private alleles (P_a) per group.
*Significant deviation from random mating expectations.

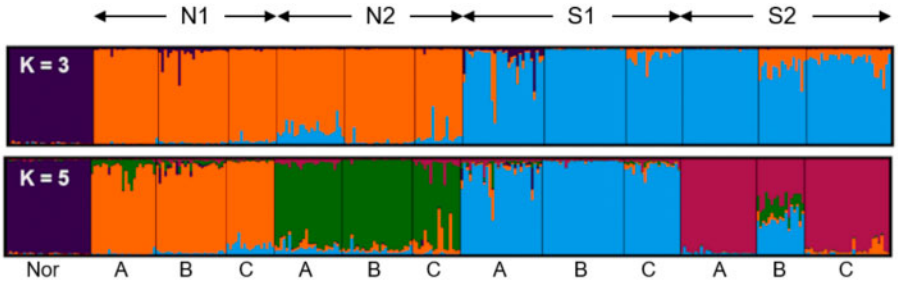

Figure 2. Genetic subdivision of *Laminaria digitata* based on STRUCTURE analysis. K1–13 were explored, and K3 and K5 represent the two most likely number of clusters based on ΔK outlier identification.

each of the four regions (S2: $F_{ST} = 0.13$; $P < 0.001$; S1: $F_{ST} = 0.032$, $P = 0.001$; N2: $F_{ST} = 0.023$, $P = 0.003$; N1: $F_{ST} = 0.032$, $P = 0.001$). Across all samples, F_{ST} was 0.174 ($P < 0.0001$), all pairwise F_{ST} values were significant (Table 2), and there was a significant correlation between pairwise F_{ST} and geographical distance (Figure 3). In line with the STRUCTURE results at $K = 2$ pairwise, F_{ST} supported the strong differentiation of the Southern (S1 + 2) from Northern (N1 + 2) region and Norway sample (Figure 4). The results were almost identical after null allele correction. AMOVA grouping samples according to Southern or Northern region revealed a higher level of differentiation between these groups ($F_{CT} = 0.114$) than within groups ($F_{SC} = 0.093$). AMOVA for regions separately supported a higher level of differentiation between regions and among sites within regions for the Southern region ($F_{CT} = 0.09$, $P < 0.0001$; $F_{SC} = 0.08$, $P < 0.0001$) compared to the Northern region ($F_{CT} = -0.01$, $P = 0.932$; $F_{SC} = 0.06$, $P < 0.0001$). Permutation analysis also revealed the overall F_{ST} for the Southern region ($F_{ST} = 0.131$) to be significantly higher ($P < 0.01$) than that for the Northern region (F_{ST} excluding N1 = 0.056).

Genetic diversity

Applying the same group-based permutation analysis also revealed that the Southern samples had significantly lower levels of within-sample variation compared to the Northern samples as measured by allelic richness (S = 2.8, N = 4.2, $P = 0.003$), observed heterozygosity (S = 0.39, N = 0.51, $P = 0.009$), and relatedness (S = 0.212, N = 0.09, $P < 0.001$). This trend was also apparent when samples were analysed separately, with a clear decline in variability reported by indices of diversity moving down from the Northern to Southern region (Table 1 and Figure 5). The Norwegian sample disrupted this inverse latitudinal cline, most obviously in the level of mean intrasample relatedness (Figure 5 and Supplementary Figure S1), compared to the Northern samples. The highest number of private alleles was

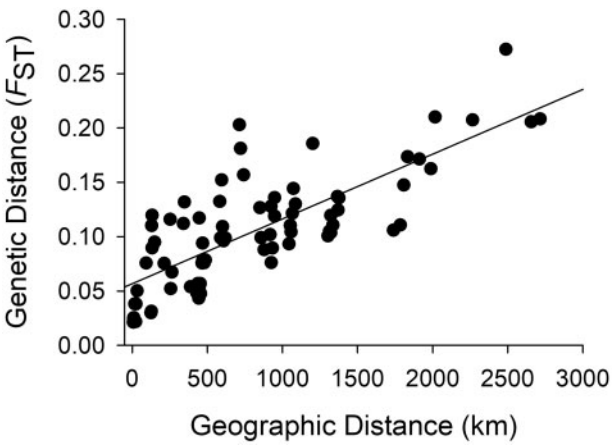

Figure 3. Mantel tests result showing significant ($P < 0.001$) correlation between geographical distance (km) and genetic distance (FST) between pairs of samples.

Table 2. Pairwise F_{ST} values between samples (below diagonal) with associated *P* values of significance estimated after 10 000 permutations (upper diagonal).

	x1 Nor	N1-A	N1-B	N1-C	N2-A	N2-B	N2-C	S1-A	S1-B	S1-C	S2-A	S2-B	S2-C
Nor	–	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
N1-A	0.104	–	0.005	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
N1-B	0.089	0.024	–	0.015	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
N1-C	0.111	0.038	0.025	–	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
N2-A	0.111	0.057	0.052	0.057	–	0.038	0.001	0.001	0.001	0.001	0.001	0.001	0.001
N2-B	0.106	0.048	0.048	0.043	0.022	–	0.001	0.001	0.001	0.001	0.001	0.001	0.001
N2-C	0.147	0.079	0.076	0.078	0.050	0.038	–	0.001	0.001	0.001	0.001	0.001	0.001
S1-A	0.162	0.102	0.089	0.076	0.078	0.076	0.116	–	0.002	0.001	0.001	0.001	0.001
S1-B	0.210	0.144	0.121	0.104	0.098	0.109	0.151	0.030	–	0.035	0.001	0.001	0.001
S1-C	0.207	0.130	0.110	0.093	0.096	00.098	0.132	0.031	0.021	–	0.001	0.001	0.001
S2-A	0.272	0.186	0.171	0.174	0.157	0.181	0.203	0.116	0.120	0.110	–	0.001	0.001
S2-B	0.206	0.119	0.105	0.101	0.088	0.099	0.126	0.054	0.068	0.052	0.095	–	0.001
S2-C	0.208	0.135	0.124	0.137	0.119	0.136	0.127	0.094	0.132	0.112	0.075	0.076	–

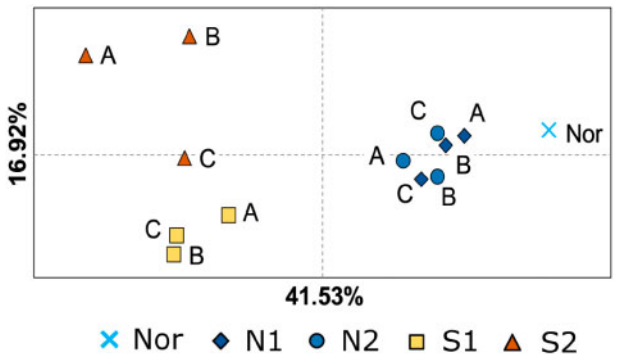

Figure 4. Principal coordinate analysis of pairwise *FST* values.

detected for the N1 region. Interestingly, S2, despite showing the lowest levels of variation, revealed a larger number of private alleles than either S1 or N2 (Table 1).

Comparison with data from Robuchon et al. (2014) revealed within-sample variation to be lower among populations in France than among Northern populations (N1 and N2 grouped) when quantified using allelic richness (France $A_r = 4.4$; Northern $A_r = 6.7$; $P = 0.02$) and relatedness (France $r = 0.176$; Northern $r = 0.051$; $P < 0.001$), while intersample differentiation was greater in France than in our Northern region (France $F_{ST} = 0.103$; Northern $F_{ST} = 0.03$; $P = 0.001$). F_{IS} was significantly larger in the Northern region than in France (France $F_{IS} = 0.069$; Northern $F_{IS} = 0.224$; $P = 0.001$). French samples did not differ significantly from samples from our Southern region (S1 and S2 grouped) for any these variables.

Discussion

Genetic studies on *L. digitata* have largely been restricted to southern, trailing-edge populations found towards the equatorward extreme of its distribution. Although a previous study (Brennan et al., 2014) analysed local gene flow patterns around Lough Neagh (Northern Ireland, UK), our study is the first to assess this species' genetic structure at multiple spatial scales and across both southern trailing-edge and northern range-centre populations. The data revealed a hierarchical structure including features such as a macrogeographic correlation between genetic and geographical distances, and fine-scale differentiation, similarly reported in studies of French populations (Billot et al., 2003; Robuchon et al., 2014). A key finding was less variation within, and greater differentiation among, southern populations compared to those in the north. This pattern was consistent after the integration of data from Robuchon et al. (2014), which revealed variation in northern France to be similar to our southern UK populations.

Equatorward trailing-edge populations of poleward shifting species have been highlighted as of special conservation significance as many occur within low-latitude areas that were formerly glacial refugia. This means that they often harbour a large proportion of a species' genetic variation and/or unique lineages (Provan and Maggs, 2012). In this context, the low genetic variability in trailing-edge *L. digitata* populations on both UK and French coastlines is striking as these areas flank the well-known Hurd deep glacial refuge. Phylogeographic studies have shown this to be a reservoir of relic variation for a number of macrophytes (Coyer et al., 2003; Provan et al., 2005; Hoarau et al., 2007). It is likely this area played a similar role for *L. digitata* as the considerably larger number of private alleles among S2 populations, compared to the more northern S1 populations, is compatible with a glacial refuge in the English Channel area. Robuchon et al. (2014) reported similar decreases in the number of private alleles in samples moving away from the English Channel towards the absolute range edge of *L. digitata* in southern Brittany. In light of this and expectations of refugial richness, the present low diversity must be considered a reduction from historically higher levels. This reduction is most likely due to increased drift from a combination of low-quality habitat, lower effective population size, and greater isolation associated with persistence at the trailing edge (Eckert et al., 2008).

There have been few studies investigating the phylogeography of Arctic and cold-temperate seaweeds. Most inferences of glacial refugia have been inferred from species with warm-water affinities that penetrate into waters that remained ice-free during glacial maxima. For *L. digitata*, the higher levels of variation and private allele numbers for Northern vs. Southern regions and within N1 vs. N2 are compatible with a northern glacial refuge and are contrary to expectations of sequential founder effect patterns during colonization from a southern refuge. Assis et al. (2018a) generated hind-casted models of suitable areas for persistence during glacial maxima for North Atlantic kelp. For *L. digitata*, and cold-temperate and Arctic kelp more generally, the Faroe Islands and southern Iceland were identified as potential northern refugia. This is supported by phylogeographic studies on the arctic intertidal fucoid, *Fucus distichus* (Coyer et al., 2011; Neiva et al., 2016) and

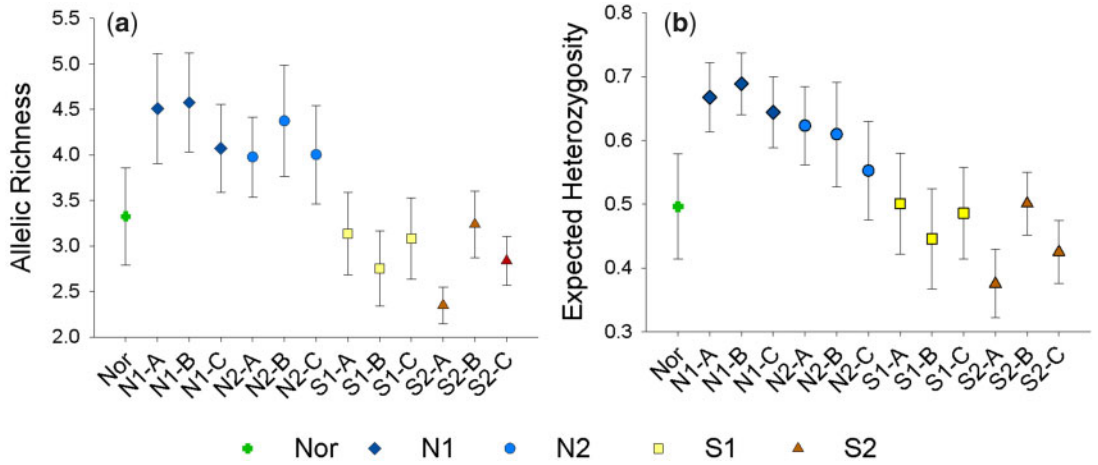

Figure 5. Mean (± 1 SE): (a) allelic richness and (b) expected heterozygosity for sampled *Laminaria digitate* sites.

studies on plants, birds, and marine invertebrates (Aegisdóttir and Thórhallsdóttir, 2004; Maggs et al., 2008; Ingólfsson, 2009). However, to confirm the location, number, and contribution of northern glacial refugia for *L. digitata*, further range-wide sampling is required. This will also allow a greater understanding of pan Atlantic recolonization pathways as *L. digitata* may also have survived in southern glacial refugia in the NW Atlantic (Assis et al., 2018a; Bringloe and Saunders, 2018).

Our study reported a significant correlation between genetic separation and geographic distances across a broad spatial scale, as has been observed in previous smaller-scale studies of *L. digitata* (Billot et al., 2003; Brennan et al., 2014; Robuchon et al., 2014) and other macrophytes (Durrant et al., 2014). However, this pattern does not necessarily indicate a direct link between recurrent connectivity and geographical distance *per se* for two main reasons. First, genetic structure may reflect historical rather than contemporary connectivity patterns, as has been demonstrated for European populations of the kelp *Saccharina latissima* (Luttikhuizen et al., 2018). Second, correlations with geographical distance may be detected despite more important contributions of other seascape factors. For example, Durrant et al. (2018) reported a more important role of habitat availability than geographical distance despite a significant correlation between geographical and genetic distances. Similar seascape effects could explain the larger genetic differences between Southern and Northern regions, separated by an area of interrupted rocky substratum, compared to N1 vs. N2 despite the similar intervening geographical distances. This regional genetic differentiation may also reflect restricted secondary contact between populations emanating from southern and northern glacial refugia, as suggested by Hoarau et al. (2007).

With the exception of S2-A, all samples reported significant heterozygote deficits (positive F_{IS} values). This differs from the general conformance to Hardy–Weinberg proportions in French populations (Billot et al., 2003; Robuchon et al., 2014). Technical artefacts such as null alleles can be discounted as the same loci as Robuchon et al. (2014) were employed. The detection of similar heterozygotes in the Lough Neagh based study by Brennan et al. (2014) points to biological drivers of the observed patterns. Such biological drivers can include selection, inbreeding, and Wahlund's effect. Selection effects at multiple loci are unlikely as microsatellites are typically selectively neutral. Inbreeding has been detected in small kelp populations (Luttikhuizen et al., 2018) but can be ruled out in the case of the Northern samples, given that levels of relatedness were similar to expectations of a panmictic population. Wahlund's effect is commonly reported explanation for heterozygote deficits. This occurs where a sample comprises individuals from more than one genetically differentiated group. In this case, Wahlund's effect could be due to any combination of fine-scale population subdivision (Jorde et al., 2018), temporal differences among cohorts (McKeown et al., 2017), or sampling of multiple kin groups (Castric and Bernatchez, 2003). Disentangling the exact type will require the fine-scale analysis of cohort and georeferenced individuals. However, the low dispersal capacity of seaweeds in general (Santelices, 1990) and the density barrier effects of infrequent dispersal into saturated systems (Neiva et al., 2012; Waters et al., 2013) would be expected to restrict mixing and increase variances in reproductive success contributing to spatial/temporal Wahlund's effects. It seems the respective roles of inbreeding and Wahlund's effect likely vary throughout *L. digitata*'s range but further study will be needed to assess if these are linked to regional differences in the species' reproductive ecology or environmental (e.g. seascape) effects.

The positive relationship between genetic diversity and population resilience is conceptually well understood, but empirical data exploring this link are lacking for non-model species (Reusch, 2003; Hughes and Stachowicz, 2004). Here, we explore the link between neutral genetic diversity and resilience characteristics, as the same sites analysed here were included in the common garden temperature experiments by King et al. (2019). King et al. (2019) demonstrated that individuals in northern populations, identified here as being more genetically variable, were less able to tolerate thermal insults than individuals in southern populations, which were less diverse. This contrasts with the results of Wernberg et al. (2018) who found a positive relationship between resilience to climatic stress (heatwaves) and intra-population genetic variation. An important difference between both systems is that for *L. digitata* there may be a higher level of pre-existing local adaptation to thermal regimes (King et al., 2018, 2019; Wernberg et al., 2018) associated with the strong selection at trailing-edge populations (Kawecki, 2008). Such adaptive selection is undetected by neutral markers. Therefore, levels of neutral genetic diversity *per se* may be a poor predictor of resilience to thermal stress in this system as neutral genetic diversity

may be misaligned with spatial patterns of local adaptation that are more important factors underpinning such resilience.

Cool-water kelps have been under-represented in phylogeographic studies to date. This study offers insight into how cool-water kelps may have persisted in disjunct glacial refugia during glacial maxima. In the NE Atlantic, much of *L. digitata*'s southern extent is predicted to disappear over the coming decades (Raybaud *et al.*, 2013; Assis et al., 2018a). This study indicates that considerable ancestral genetic variation has likely already been lost at the trailing edge. While the conservation value of such trailing-edge populations is debated, in the case of *L. digitata*, it is indisputable, as these populations harbour advantageous adaptations for climate change resilience. There is also increased interest in expanding commercial exploitation of *L. digitata* throughout its distribution. The fine-scale differentiation reported here demonstrates that management of harvested populations must be on a local scale to ensure sustainability. In light of coarse patterns of neutral genetic structure and local adaptation resolved here and by King et al. (2019), species-wide and more local conservation and management plans would benefit from a seascape genomics approach to disentangle the respective influences of demographics, adaptation, and plasticity on individual- and population-level fitness.

Data archiving

Microsatellite genotypes are available at: 10.6084/m9.figshare.11967714.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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References

Aegisdóttir, H. H., and Thórhallsdóttir, T. E. 2004. Theories on migration and history of the North-Atlantic flora: a review. Jökull, 54: 1–16.

Almany, G. R., Connolly, S. R., Heath, D. D., Hogan, J. D., Jones, G. P., McCook, L. J., Mills, M., *et al.* 2009. Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. Coral Reefs, 28: 339–351.

Assis, J., Araujo, M. B., and Serrao, E. A. 2018a. Projected climate changes threaten ancient refugia of kelp forests in the North Atlantic. Global Change Biology, 24: E55–E66.

Assis, J., Serrao, E. A., Coelho, N. C., Tempera, F., Valero, M., and Alberto, F. 2018b. Past climate changes and strong oceanographic barriers structured low-latitude genetic relics for the golden kelp *Laminaria ochroleuca*. Journal of Biogeography, 45: 2326–2336.

Beaumont, N., Austen, M., Mangi, S., and Townsend, M. 2008. Economic valuation for the conservation of marine biodiversity. Marine Pollution Bulletin, 56: 386–396.

Billot, C., Engel, C. R., Rousvoal, S., Kloareg, B., and Valero, M. 2003. Current patterns, habitat discontinuities and population genetic structure: the case of the kelp *Laminaria digitata* in the English Channel. Marine Ecology Progress Series, 253: 111–121.

Brennan, G., Kregting, L., Beatty, G. E., Cole, C., Elsäßer, B., Savidge, G., and Provan, J. 2014. Understanding macroalgal dispersal in a complex hydrodynamic environment: a combined population genetic and physical modelling approach. Journal of the Royal Society Interface, 11:20140197.

Bringloe, T. T., and Saunders, G. W. 2018. Mitochondrial DNA sequence data reveal the origins of postglacial marine macroalgal flora in the Northwest Atlantic. Marine Ecology Progress Series, 589: 45–58.

Castric, V., and Bernatchez, L. 2003. The rise and fall of isolation by distance in the anadromous brook charr (*Salvelinus fontinalis* Mitchill). Genetics, 163: 983–996.

Chapuis, M. P., and Estoup, A. 2007. Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution, 24: 621–631.

Coleman, M. A., Cetina-Heredia, P., Roughan, M., Feng, M., Sebille, E., and Kelaher, B. P. 2017. Anticipating changes to future connectivity within a network of marine protected areas. Global Change Biology, 23: 3533–3542.

Coyer, J. A., Hoarau, G., Van Schaik, J., Luijckx, P., and Olsen, J. L. 2011. Trans-Pacific and trans-Arctic pathways of the intertidal macroalga *Fucus distichus* L. reveal multiple glacial refugia and colonizations from the North Pacific to the North Atlantic. Journal of Biogeography, 38: 756–771.

Coyer, J. A., Peters, A. F., Stam, W. T., and Olsen, J. L. 2003. Post-ice age recolonization and differentiation of *Fucus serratus* L. (Phaeophyceae; Fucaceae) populations in Northern Europe. Molecular Ecology, 12: 1817–1829.

Diez, I., Muguerza, N., Santolaria, A., Ganzedo, U., and Gorostiaga, J. M. 2012. Seaweed assemblage changes in the eastern Cantabrian Sea and their potential relationship to climate change. Estuarine Coastal and Shelf Science, 99: 108–120.

Durrant, H. M. S., Barrett, N. S., Edgar, G. J., Coleman, M. A., and Burridge, C. P. 2018. Seascape habitat patchiness and hydrodynamics explain genetic structuring of kelp populations. Marine Ecology Progress Series, 587: 81–92.

Durrant, H. M. S., Burridge, C. P., Kelaher, B. P., Barrett, N. S., Edgar, G. J., and Coleman, M. A. 2014. Implications of macroalgal isolation by distance for networks of marine protected areas. Conservation Biology, 28: 438–445.

Eckert, C. G., Samis, K. E., and Lougheed, S. C. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Molecular Ecology, 17: 1170–1188.

Eggert, A. 2012. Seaweed responses to temperature. In Seaweed Biology, pp. 47–66. Ed. By C. Wiencke, and K. Kischof. Springer, Berlin, Germany.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14: 2611–2620.

Excoffier, L., and Lischer, H. E. L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10: 564–567.

Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes - application to human mitochondrial-DNA restriction data. Genetics, 131: 479–491.

Goudet, J. 1995. FSTAT (Version 1.2): a computer program to calculate F-statistics. Journal of Heredity, 86: 485–486.

Heino, M., Kaitala, V., Ranta, E., and Lindstrom, J. 1997. Synchronous dynamics and rates of extinction in spatially structured populations. Proceedings of the Royal Society B: Biological Sciences, 264: 481–486.

Hereward, H. F. R., King, N. G., and Smale, D. A. 2020. Intra-annual variability in responses of a canopy forming kelp to cumulative low tide heat stress: implications for populations at the trailing edge. Journal of Phycology, 56: 146–158.

Hewitt, G. M. 1999. Post-glacial recolonisation of European biota. Biological Journal of the Linnean Society, 68: 87–112.

Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences, 359: 183–195.

Hoarau, G., Coyer, J. A., Veldsink, J. H., Stam, W. T., and Olsen, J. L. 2007. Glacial refugia and recolonization pathways in the brown seaweed *Fucus serratus*. Molecular Ecology, 16: 3606–3616.

Hubisz, M. J., Falush, D., Stephens, M., and Pritchard, J. K. 2009. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources, 9: 1322–1332.

Hughes, A. R., and Stachowicz, J. J. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proceedings of the National Academy of Sciences of the United States of America, 101: 8998–9002.

Ingólfsson, A. 2009. A marine refugium in Iceland during the last glacial maximum: fact or fiction? Zoologica Scripta, 38: 663–665.

Jorde, P. E., Andersson, A., Ryman, N., and Laikre, L. 2018. Are we underestimating the occurrence of sympatric populations? Molecular Ecology, 27: 4011–4025.

Kawecki, T. J. 2008. Adaptation to marginal habitats. Annual Review of Ecology, Evolution, and Systematics, 39: 321–342.

King, N. G., McKeown, N. J., Smale, D. A., and Moore, P. J. 2018. The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. Ecography, 41: 1469–1484.

King, N. G., McKeown, N. J., Smale, D. A., Wilcockson, D. C., Hoelters, L., Groves, E. A., Stamp, T., *et al.* 2019. Evidence for different thermal ecotypes in range centre and trailing edge kelp populations. Journal of Experimental Marine Biology and Ecology, 514–515: 10–17.

Krause-Jensen, D., and Duarte, C. M. 2016. Substantial role of macroalgae in marine carbon sequestration. Nature Geoscience, 9: 737–742.

Krumhansl, K. A., Okamoto, D. K., Rassweiler, A., Novak, M., Bolton, J. J., Cavanaugh, K. C., Connell, S. D., *et al.* 2016. Global patterns of kelp forest change over the past half-century. Proceedings of the National Academy of Sciences of the United States of America, 113: 13785–13790.

Luttikhuizen, P. C., van den Heuvel, F. H. M., Rebours, C., Witte, H. J., van Bleijswijk, J. D. L., and Timmermans, K. 2018. Strong population structure but no equilibrium yet: genetic connectivity and phylogeography in the kelp *Saccharina latissima* (Laminariales, Phaeophyta). Ecology and Evolution, 8: 4265–4277.

Maggs, C. A., Castilho, R., Foltz, D., Henzler, C., Jolly, M. T., Kelly, J., Olsen, J., *et al.* 2008. Evaluating signatures of glacial refugia for north Atlantic benthic marine taxa. Ecology, 89: S108–S122.

McKeown, N. J., Hauser, L., and Shaw, P. W. 2017. Microsatellite genotyping of brown crab *Cancer pagurus* reveals fine scale selection and 'non-chaotic' genetic patchiness within a high gene flow system. Marine Ecology Progress Series, 566: 91–103.

Neiva, J., Pearson, G. A., Valero, M., and Serrao, E. A. 2012. Fine-scale genetic breaks driven by historical range dynamics and ongoing density-barrier effects in the estuarine seaweed *Fucus ceranoides*. BMC Evolutionary Biology, 12: 78.

Neiva, J., Serrão, E. A., Assis, J., Pearson, G. A., Coyer, J. A., Olsen, J. L., Hoarau, G., et al. 2016. Climate Oscillations, Range Shifts and Phylogeographic Patterns of North Atlantic Fucaceae . *In* Seaweed Phylogeography, pp. 279–308. Ed. By ZM. Hu and C. Fraser. Springer, Dordrecht.

Nicastro, K. R., Zardi, G. I., Teixeira, S., Neiva, J., Serrao, E. A., and Pearson, G. A. 2013. Shift happens: trailing edge contraction associated with recent warming trends threatens a distinct genetic lineage in the marine macroalga *Fucus vesiculosus*. BMC Biology, 11: 6.

Peakall, R., and Smouse, P. E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6: 288–295.

Pielou, E. C. 1991. After the Ice Age: the return of life to glaciated North America. University of Chicago Press, Chicago, IL, USA

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945–959.

Provan, J., and Maggs, C. A. 2012. Unique genetic variation at a species' rear edge is under threat from global climate change. Proceedings of the Royal Society B: Biological Sciences, 279: 39–47.

Provan, J., Wattier, R. A., and Maggs, C. A. 2005. Phylogeographic analysis of the red seaweed *Palmaria palmata* reveals a Pleistocene marine glacial refugium in the English Channel. Molecular Ecology, 14: 793–803.

Raybaud, V., Beaugrand, G., Goberville, E., Delebecq, G., Destombe, C., Valero, M., Davoult, D., *et al.* 2013. Decline in kelp in west Europe and climate. PLoS One, 8: e66044.

Reusch, T. B. H. 2003. Floral neighbourhoods in the sea: how floral density, opportunity for outcrossing and population fragmentation affect seed set in Zostera marina. Journal of Ecology, 91: 610–615.

Robuchon, M., Le Gall, L., Mauger, S., and Valero, M. 2014. Contrasting genetic diversity patterns in two sister kelp species co-distributed along the coast of Brittany, France. Molecular Ecology, 23: 2669–2685.

Rousset, F. 2008. GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources, 8: 103–106.

Santelices, B. 1990. Patterns of reproduction, dispersal and recruitment in seaweeds. Oceanography and Marine Biology: An Annual Review, 28: 177276.

Smale, D. A. 2020. Impacts of ocean warming on kelp forest ecosystems. New Phytologist, 225: 1447–1454.

Smale, D. A., Burrows, M. T., Evans, A. J., King, N., Sayer, M. D. J., Yunnie, A. L. E., and Moore, P. J. 2016. Linking environmental variables with regional-scale variability in ecological structure and standing stock of carbon within UK kelp forests. Marine Ecology Progress Series, 542: 79–95.

Smale, D. A., Burrows, M. T., Moore, P., O'Connor, N., and Hawkins, S. J. 2013. Threats and knowledge gaps for ecosystem services provided by kelp forests: a northeast Atlantic perspective. Ecology and Evolution, 3: 4016–4038.

Steneck, R. S., Graham, M. H., Bourque, B. J., Corbett, D., Erlandson, J. M., Estes, J. A., and Tegner, M. J. 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. Environmental Conservation, 29: 436–459.

Teagle, H., Hawkins, S. J., Moore, P. J., and Smale, D. A. 2017. The role of kelp species as biogenic habitat formers in coastal marine ecosystems. Journal of Experimental Marine Biology and Ecology, 492: 81–98.

Valero, M., Destombe, C., Mauger, S., Ribout, C., Engel, C. R., Daguin-Thiebaut, C., and Tellier, F. 2011. Using genetic tools for sustainable management of kelps: a literature review and the example of *Laminaria digitata*. Cahiers de Biologie Marine, 52: 467–483.

Waters, J. M., Fraser, C. I., and Hewitt, G. M. 2013. Founder takes all: density-dependent processes structure biodiversity. Trends in Ecology and Evolution, 28: 78–85.

Webb, T., and Bartlein, P. J. 1992. Global changes during the last 3 million years: climatic controls and biotic response. Annual Review of Ecology and Systematics, 23: 141–173.

Wernberg, T., Coleman, M. A., Bennett, S., Thomsen, M. S., Tuya, F., and Kelaher, B. P. 2018. Genetic diversity and kelp forest vulnerability to climatic stress. Scientific Reports, 8: 1851.

Wernberg, T., Smale, D. A., Tuya, F., Thomsen, M. S., Langlois, T. J., de Bettignies, T., Bennett, S., *et al.* 2013. An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. Nature Climate Change, 3: 78–82.

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