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Broad scale agreement between intertidal habitats and adaptive traits on a basis of contrasting population genetic structure

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ABSTRACT

Understanding the extent to which neutral processes and adaptive divergence shape the spatial structure of natural populations is a major goal in evolutionary biology and is especially important for the identification of significant levels of biodiversity. Our results identified replicated habitat-specific (adaptive) phenotypic divergence in the brown macroalga *Fucus vesiculosus* that is independent of population (neutral) genetic structure. *F. vesiculosus* inhabits contiguous and contrasting marine to estuarine intertidal habitats. Combining analyses of genetic and phenotypic traits of populations living under differential selective regimes (estuaries and open coast), we investigated levels of neutral genetic differentiation and adaptive physiological responses to emersion stress. In southwest England (SW UK) and northern Iberia (N. Iberia), populations living in estuaries and marine coastal habitats were genetically characterized at six microsatellite loci. In N. Iberia, two clades with limited admixture were recovered, each including one open coast site and the adjacent estuarine location. In contrast, SW UK samples clustered according to habitat and formed three distinct groups of genotypes; one including the two open coast locations and the other two representing each of the estuarine sites. Temperature loggers revealed distinct emersion regimes that characterized each habitat type independently of the region, while water and air temperature profiles showed site-specific trends. Despite acclimation under usual conditions, trait means of emersion stress resilience showed a strong phenotypic divergence between habitats, consistent with environmental clines in exposure time observed in the different habitats. We demonstrate that neutral genetic clusters do not reflect locally adapted population units. Our results identified replicated habitat-specific (adaptive) phenotypic divergence that is independent of population (neutral) genetic structure in *F. vesiculosus*. The significance of such findings extends beyond the theoretical evolutionary and ecological interest of discovering parallel adaptive responses to the broader implications for conservation of intraspecific biodiversity.

1. Introduction

A central aim in evolutionary biology is to understand the relative roles of neutral and adaptive forces in shaping the spatial structure of natural populations (McKay and Latta, 2002; Winker, 2009). When investigating evolutionarily significant levels of biodiversity it is especially important to complement approaches based on neutral genetic markers, conventionally utilized to infer demographic connectivity, with methods that can also provide insights into adaptive dynamics across heterogeneous habitats (e.g. Ballentine and Greenberg, 2010; Mariani et al., 2012). Reliance on neutral molecular markers to identify evolutionary significant units, while ignoring phenotypic divergence between populations that lack detectable genetic divergence, will result in underrepresentation of locally adapted populations and underestimation of biodiversity, potentially misleading conservation efforts (Zink, 2004; Phillimore and Owens, 2006).

Geographically widespread species often display distinct subgroups which may be adapted to their local environment (Zardi et al., 2011b). Differences in local environments may result in spatially divergent selective regimes, which can lead to rapid local adaptation across populations of the same species in the absence of divergence in neutral molecular markers (Crispo, 2008; Teske et al., 2013). Indeed, increasing studies report significant intraspecific phenotypic divergence between populations inhabiting distinct habitats despite the lack of divergence detected with neutral DNA markers (Funk and Omland, 2003; Lin et al., 2008; Ballentine and Greenberg, 2010).

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Populations of species that occupy adjacent and contrasting environments present distinct advantages for studying the interplay between local adaptation and genetic structure due to neutral processes, as contrasting environments can create divergent selection pressures, while close proximity maintains the opportunity for gene flow. A good example in marine intertidal habitats is the contrast between open coasts and adjacent estuaries, forming clines in which abiotic environmental conditions change rapidly over a few hundreds of metres (Boyden et al., 1979; Hilbish et al., 2003). In general, a continuum of marine intertidal assemblages exists from the open coast to the inner estuarine habitats, with intraspecific phenotypic shifts in response to environmental changes, typifying plastic phenotypic variations or genetically distinct ecotypes (e.g. Attrill and Rundle, 2002). Transitional estuarine environments can also create habitat breaks and dispersal barriers that could enhance divergence and *in situ* speciation of organisms with truly estuarine origins, rather than simply estuarine ecotypes of marine species (e.g. Bilton et al., 2002; Kelly et al., 2006).

By occupying both open coast and estuarine intertidal habitats, the brown seaweed *Fucus vesiculosus* is an attractive model organism to study patterns of intraspecific divergence. *Fucus vesiculosus* reproduces sexually and gamete dispersal is restricted (Serrão et al., 1997). The drifting of dislodged reproductive fragments (rafting) could mediate long range dispersal (Muhlin et al., 2008), although effective gene flow may be reduced/prevented by priority colonization effects (Neiva et al., 2012). Thus, tidal transport of rafting individuals could maintain genetic connectivity among populations of *F. vesiculosus* from the open coast and adjacent estuaries, while strong disruptive selection along these environmental clines could adaptively maintain distinct ecotypes and prevent homogenization.

Here we examine the relationship between neutral genetic and adaptive phenotypic differentiation in the contemporary evolution of *Fucus vesiculosus* populations inhabiting contrasting intertidal habitats. In particular, the following hypotheses were tested: 1) genetic structure and diversity between open coast populations is lower than that between estuarine populations; 2) populations inhabiting each habitat (open coast *vs*. estuarine) are phenotypically differentiated, and 3) potentially selective thermal regimes differ between habitats. To test these hypotheses, we investigated spatial population structure in *F. vesiculosus* populations occupying contiguous and distinct open coast and estuarine intertidal habitats using putatively neutral microsatellite markers unaffected by natural selection. We then estimated trait means of emersion stress resilience in individuals from the two habitats in a common laboratory environment (common garden) Finally, we compared maximum emersion times (upper vertical limits) and summer thermal regimes from *in situ* temperature profiles experienced in the field by populations from the two habitats.

2. Materials and methods

Fucus vesiculosus individuals were collected from southwest England (hereafter SW UK) and from north-west Iberia (hereafter N. Iberia). In each region, two open coast (Bream Cove and Paignton in SW UK, A Guarda and Viana do Castelo in N. Iberia) and two innermost estuarine populations (Scott's Quay and Sharpham in SW UK, Rio Minho and Rio Lima in N. Iberia) were selected (Fig. 1). At each site, environmental temperatures and exposure times were recorded and vegetative apices from 50 individuals were sampled and stored in silica drying crystals for genetic analyses. Haphazard subsets of these individuals (20 per site) were brought to the laboratory for experimental tests of physiological resilience to emersion.

2.1. Environmental temperatures and exposure times

Temperature data were collected at estuarine and open coast locations in 2009 between May 26th and September 18th using dataloggers (iButtons®, Maxim Integrated Products, Dallas Semiconductor, USA) fixed in the mid intertidal distributional range of *Fucus vesiculosus*. Dataloggers were placed inside protective brass housings, sealed with o-rings and silicon grease, and glued flush to the chiseled rock surface using fast-curing epoxy (Z-Spar Splash-zone compound). Thermal data from rock surfaces are practical proxies for thallus temperatures of desiccating algae (Pearson et al., 2009). Data from 4 to 6 replicate loggers at each site were collected at 90 min intervals. Values from the replicate loggers were averaged and water temperature at high tide and air temperature during low tide were calculated for each day at each site. A second set of dataloggers was deployed at the high shore limit of *F. vesiculosus* at each site. Data from three replicate loggers per location were collected at 10 min intervals between May 25th and June 8th. Values from the three loggers were averaged and used to estimate emersion times experienced by the populations.

2.1.1. Data analyses

Emersion time data fulfilled the pre-requisites for parametric analysis (Cochran's Test) and were analysed using GMAV5 software (Underwood et al., 2002). Air and water temperature data did not meet these assumptions and therefore they were analysed using the PERMANOVA module (Anderson, 2001; McArdle and Anderson, 2001). In all cases, data were analysed under a nested design with habitat (open coast, estuarine) and region (SW UK, N. Iberia) as fixed factors, and site (1, 2) nested within region. Distance-based homogeneity of dispersion tests, tests of main effects and pairwise tests on significant interactions were performed using 999 permutations.

2.2. Physiological resilience to emersion

Algae (20 individuals × 8 sites) were acclimated in seawater (17 °C) for seven days in 5 L tanks at low photosynthetic photon flux density (PPFD) of 30–50 μmol m^{-2} s^{-1} (LL) supplied by sodium vapour lamps. Following acclimation, vegetative apical tips were cut, placed in 5 L tanks and kept for an additional 10 days. Half the seawater volume was replaced every 2 days throughout the acclimation period. From each individual, four tips were selected and duplicates were exposed to air at 35 °C (i.e. maximum temperature recorded with the data loggers, ±0.5 °C) at a photosynthetic photon flux density (PPFD) of 250–300 μmol photons m^{-2} s^{-1} for either (a) 7 h, or (b) 9 h (i.e. average time of exposure recorded with the data loggers in each habitat). Control treatments were kept in seawater at 17 °C under LL. After exposure, algae were allowed to recover under control conditions. After 24 h recovery, the maximum quantum yield of Photosynthesis System II (Fv/Fm) of each tip was measured with a chlorophyll fluorometer (FMS 2, Hansatech Instruments Ltd, UK). A sustained decrease in Fv/Fm is indicative of photoinhibitory damage to photosystem II (Maxwell and Johnson, 2000), and its reduction from maximal values (0.7–0.8 in brown algae) is a sensitive and rapid screening tool for stress responses.

2.2.1. Data analyses

The results were analysed using the PERMANOVA module (Anderson, 2001; McArdle and Anderson, 2001), which does not require either normality or homoscedasticity. Data were analysed under a nested design with treatment (control, 7 h exposure, 9 h exposure), habitat (open coast, estuarine) and region (SW UK, N. Iberia) as fixed factors, and site (1, 2) nested within region. Distance-based homogeneity of dispersion tests, tests of main

Fig. 1. Map of the study area. Codes for regions and sites are given in brackets. Black dots are open coast sites.

effects and pairwise tests on significant interactions were performed using 999 permutations.

2.3. Genetic analyses

DNA was isolated from 5 to 10 mg of dried tissue with a CTAB method (Hoarau et al., 2007) but using a silica filter plate (Milipore MultiScreen HTS, FB Cat. # MSFBN6B10) instead of silica fines. The six polymorphic microsatellite loci selected were amplified on a Thermal Cycler 2720 (Applied Biosystems) using forward labelled primers. PCR reactions for loci: L20, L58, L38, L94, L78 (Engel et al., 2003) consisted of a total volume of 15 μL containing 1× GoTaq polymerase buffer (Promega) with 2 mM of $MgCl_2$, 0.03 mM of each dNTP, 0.17 μM of each Forward primer and 0.33 μM of each Reverse, 0.5U GO*Taq* Polymerase and 5 μL of diluted DNA 1:10. Amplifications were obtained using the following profile: initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 20 s, followed by 35 s at 54 °C for L20, at 55 °C for L78, L38 and L94, and 53 °C for L58, 72 °C for 40 s, and a final extension at 72 °C for 20 min. F26II (Wallace et al., 2004) was amplified using 1 uL of 1:10 diluted DNA in a total volume of 10 uL containing: 1× GoTaq polymerase buffer (Promega), 2.5 mM of MgCl2, 0.2 mM of each dNTP, 0.2 uM of Forward primer, 0.4 uM of Reverse primer and 0.5 U of GoTaq Polymerase. Amplification conditions consisted in an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 30 s at 94 °C, 35 s at 55 °C and 40 s at 72 °C, and a final extension at 72 °C for 20 min. Fragment length of PCR products were analysed on an automated sequencer ABI PRISM 3130 (Applied Byosystems) using both GeneScan 350 ROX (L20, L38, L58, L78, L94) and GeneScan 500 LIZ (F26II) standards.

2.3.1. Data analyses

Standard statistics of genetic diversity (allele richness, observed heterozygosity, Hobs; Nei's gene diversity Hexp; Nei, 1978) as well

as estimators of F_{IS} (Wright, 1969) and linkage disequilibrium were calculated using the GENETIX 4.05 software (Belkhir et al., 1996–2004) and tested for significance with 10,000 permutations. Sequential Bonferroni correction (Rice, 1989) was implemented for multiple tests. Additionally, allelic richness was estimated in all populations after normalization to a sample size of 43 (equal to the smallest sample size with all loci scored) calculated as average richness over 10,000 re-sampling using the FSTAT 2.9.3 software. Genetic differentiation (F_{ST}) was estimated between pairs of populations with the estimator θ (Weir and Cockerham, 1984), and computed with GENETIX 4.05 (Belkhir et al., 1996–2004). Significance was tested using 10,000 random permutations of the individuals between samples with a threshold adjusted using sequential Bonferroni correction for multiple comparisons. The Cavalli-Sforza and Edwards' chord distance (Cavalli-Sforza and Edwards, 1967) was computed (with GENDIST), as this measure has been shown to generate higher probabilities of obtaining the correct tree topology (Takezaki and Nei, 1996). Neighbour-joining was used to assemble the tree in NEIGHBOR with bootstrap re-sampling (10,000 replications) executed using SEQBOOT and CONSENSE. All programs are part of the software package PHYLIP 3.69 (Felsenstein, 1993). Finally, to estimate the number of genotype groups that minimize Hardy–Weinberg disequilibrium and assign individuals to them, analyses were performed using STRUCTURE 2.3.3 (Pritchard et al., 2000). The dataset included all individuals using the options to ignore population affiliation when clustering individuals, assuming independence among loci, and allowing admixture. The number of possible clusters (K) assessed was 1–9 (maximum number of populations plus one), and 20 runs of 100,000 iterations (after a burn-in period of 50,000) were carried out for each K value. The height of the modal value of distribution for the posterior probability of the data for a given K was used as an indicator of the strength of the signal detected by Structure (Evanno et al., 2005). CLUMPP software (Jakobsson and Rosenberg, 2007) was subsequently used to find the optimal alignment of the 20 replicate cluster analyses of the same K. The mean membership matrix across replicates was plotted with the program DISTRUCT (Rosenberg, 2004).

3. Results

3.1. Environmental temperatures and exposure times

During the four months of monitoring, maximum temperatures recorded by the loggers occurred during emersion (Fig. 2). Estuarine locations showed very distinct thermal conditions compared to the open coast, characterized by smaller amplitude fluctuations. Temperatures over 30 °C were relatively frequent at open coast sites while they were never recorded inside estuaries. Average maximum water temperature values were 19.4 ± 2.2 and 19.6 ± 0.9 for open coast and estuarine sites respectively. Air and water temperatures did not show a habitat or regional effect but significant differences were detected between sites [Fig. 3a and b; Tables A1 and A2 in Appendix; Habitat × Site (Region), P (perm) < 0.01].

There were clear differences in exposure times for *Fucus vesiculosus* between the two habitats. Regardless of the region (N. Iberia or SW UK), the upper distributional limit of *F. vesiculosus* in the estuaries was significantly higher (exposed for longer) than on the open coast (Fig. 3c; Table A3 in Appendix; $p < 0.001$).

3.2. Physiological resilience to air exposure

In the usual condition experiments, temperature stress reduced Fv/Fm below that of controls, which did not differ between habitats [Treatment × Habitat, P (perm) < 0.01; Table A4 in Appendix; Fig. 4]. Resilience to extreme stress (9 h exposure) was higher in estuarine than open coast habitats for both regions, but did not differ at moderate stress levels (7 h exposure). Site did not have an

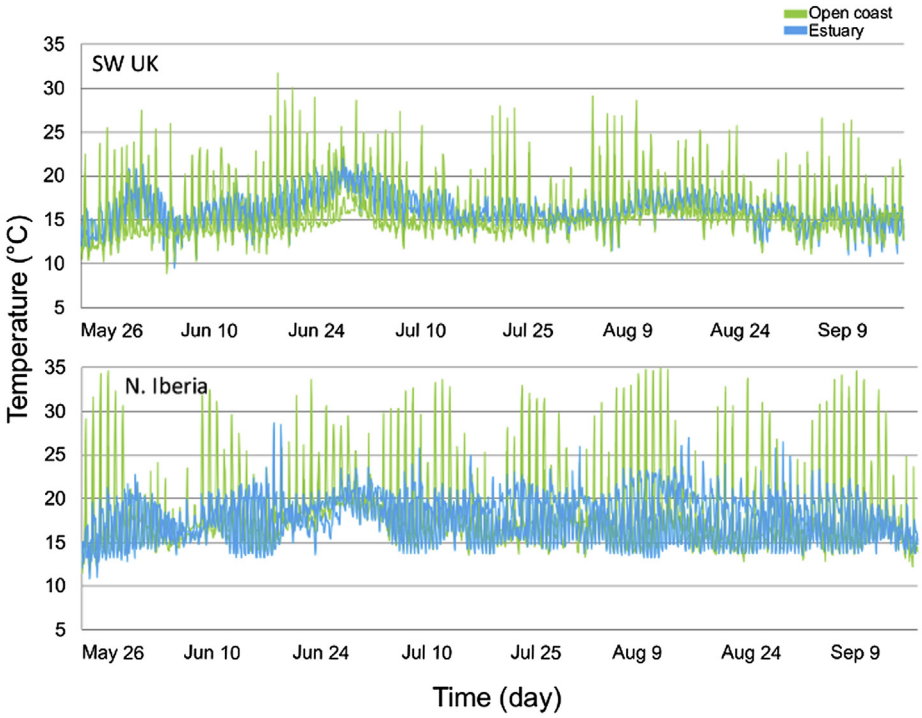

Fig. 2. Environmental temperatures. Temperature time series at the estuarine and open coast study sites (Fig. 1) from May 26th to September 18th 2009 at a) SW UK and b) N. Iberia. In each plot, each line indicates one site and represents data averaged from four to six dataloggers to illustrate the thermal range recorded at each site.

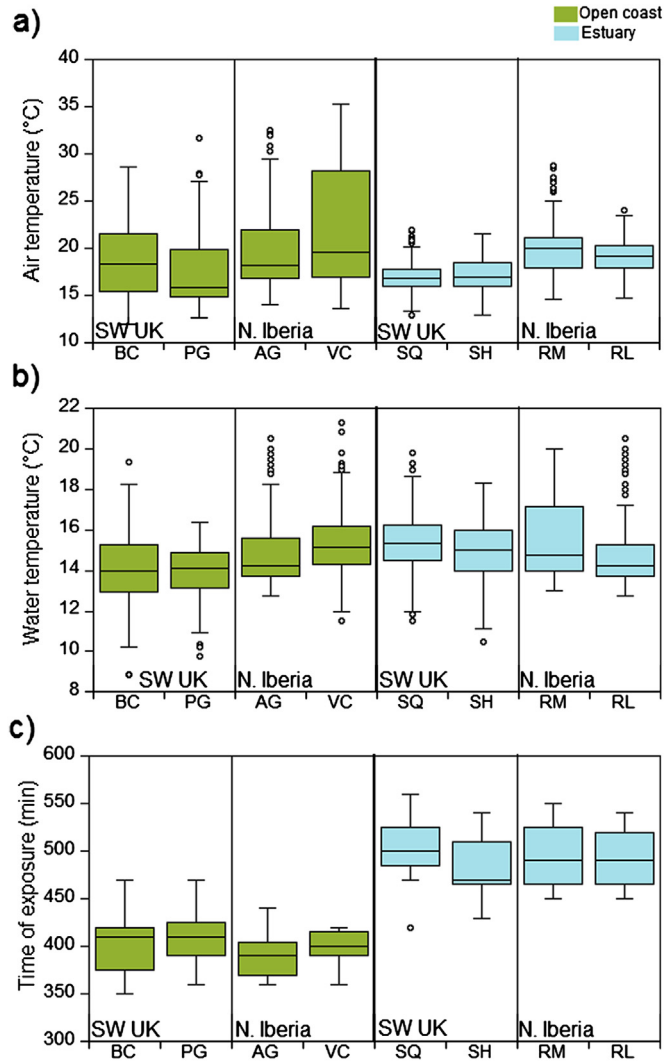

Fig. 3. Air temperatures, water temperatures and exposure times per habitat and region. Air temperatures during low tide (a), water temperatures during high tide (b) from May 26th to September 18th 2009. Exposure times (c) from May 25th to June 8th 2009. Sites within habitat and region are pooled.

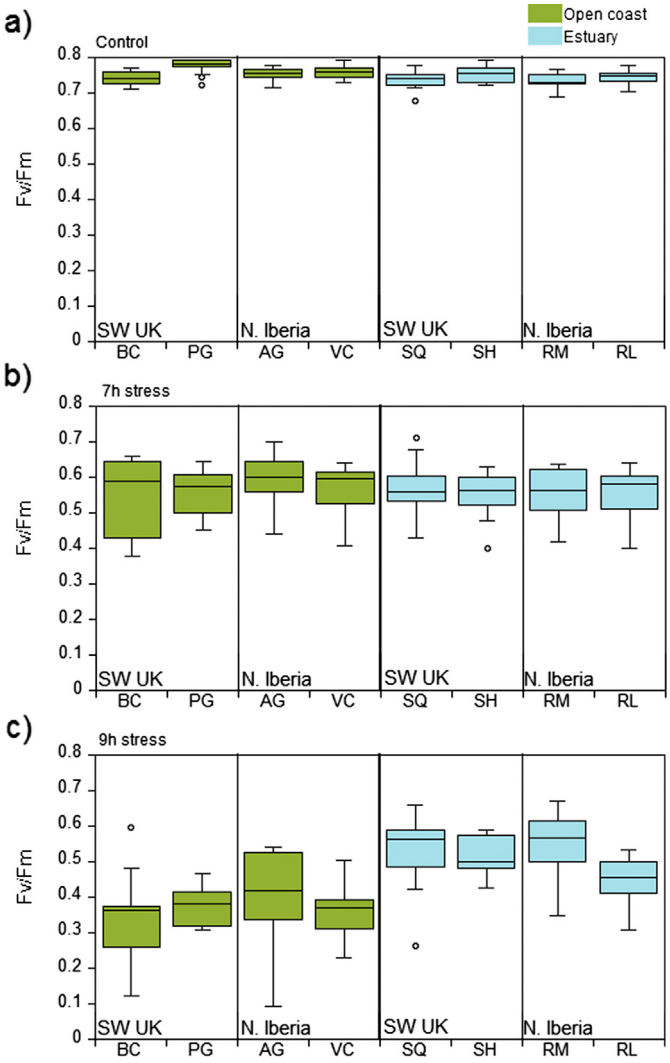

Fig. 4. Physiological resilience to air exposure. Measurements of photoinhibition of the maximum quantum yield of photosystem II (Fv/Fm) of *F. vesiculosus* (sites pooled) after air exposed at 35 °C (±0.5 °C) at a PPFD of 250–300 μmol photons·m^{-2} s^{-1} (HL) followed by 24 h of recovery: (a) controls, (b) after 7 h of air exposure, (c) after 9 h of air exposure.

effect for either region except in controls in SW UK and after 9 h of exposure in N. Iberia [Treatment × Site (Region), $p < 0.05$].

3.3. Genetic analyses

Genetic diversity was similarly high over all eight populations, both in terms of allelic richness and unbiased heterozygosity (H_{exp}; Table A5 in Appendix). From the 400 individuals assayed, a total of 91 alleles were detected with the six microsatellite loci. All loci were polymorphic in all populations.

Heterozygote deficiency was detected by a significantly positive inbreeding coefficient (F_{IS}) in four samples, including all regions and habitats (BC, AG, VC, RM). The high values of F_{IS} obtained were not locus dependent, indicating the markers used did not display technical issues such as null alleles. Additionally, null alleles were not reported in previous population genetic studies using the same markers (e.g. Perrin et al., 2007; Coyer et al., 2011).

Linkage disequilibrium was not observed among the six loci for any population (10,000 permutations; all $p > 0.01$). Strong differentiation among the eight populations was found as estimated by pairwise F_{ST} values (10,000 permutations, $p < 0.01$ for all comparisons; Table 1). F_{ST} ranged between 0.03686 (PG and BC) and 0.26252 (SH and AG).

The neighbour-joining (NJ) tree revealed 4 clusters (Fig. 5a). The open coast populations in the UK (BC and PG) grouped together strongly (bootstrap = 100%), clearly separated from their respective neighbouring estuarine populations (SQ and SH), which clustered together with weaker support (bootstrap = 67%). In contrast, the other two clusters, grouped according to geographic proximity rather than habitat (N. Iberia groups in Fig. 5a), with each population from the open coast clustering with its closest estuarine neighbour (RL and VC bootstrap = 66%; AG and RM bootstrap = 70%).

In STRUCTURE, the most probable number of clusters was five (Fig. 5b). These 5 groups were the same as shown in the NJ tree (clustered based on habitat within the UK region and based on site in N. Iberia), but with an additional separation of the two estuarine habitats in the UK as distinct genetic groups. Although distinct, individuals from the SH estuary shared some genotype proportion typical of the SQ estuary.

Table 1
Genetic differentiation between pairs of populations. Codes correspond to regions and sites in Fig. 1. Genetic differentiation (FST) estimated between pairs of populations with the estimator θ (Weir and Cockerham, 1984). All values are significant at <0.001 using 10,000 permutations.

			BC	PG	AG	VC	SQ	SH	RM	RL
Open Coast	SW UK	BC								
		PG	0.037							
	N. Iberia	AG	0.207	0.254						
		VC	0.157	0.185	0.166					
Estuarine	SW UK	SQ	0.119	0.142	0.227	0.147				
		SH	0.194	0.216	0.262	0.220	0.148			
	N. Iberia	RM	0.204	0.215	0.121	0.147	0.163	0.233		
		RL	0.136	0.185	0.190	0.097	0.167	0.245	0.175	

4. Discussion

Our results showed that estuaries versus open coasts are thermally-distinct intertidal habitats, with much higher and more frequent extreme values being reached on the open coasts despite there being no differences between means. Additionally, emersion regimes in these habitats are diverse for *Fucus vesiculosus* with a significantly longer air exposure time inside estuaries. Habitat differences outweigh regional (latitudinal scale) variation, and environmental conditions prevailing in each habitat are reflected in the physiological responses of the resident populations, supporting the existence of distinct estuarine and coastal ecotypes. In contrast, the neutral genetic background showed habitat-specific differentiation only in one of the regions (SW UK). The results from this study therefore show that ecotypic differentiation is not consistently associated with, and therefore cannot be predicted by, patterns of neutral genetic population structure, which rather reflect current restrictions to gene flow integrated with past colonization history.

4.1. Phenotypic divergence

Adaptive phenotypic divergence, driven by divergent selection, can be influenced by non-selective factors such as geographic isolation and genetic drift. Spatial isolation and physiological barriers acting on genetic connectivity among populations (Cognetti and Maltagliati, 2000; Dawson et al., 2001; Bilton et al., 2002; Zardi et al., 2007) can reinforce the effects of selection under different environmental conditions (Lee, 1999; Lee and Bell, 1999; Rynearson and Armbrust, 2004; Zardi et al., 2011b). An interesting example of rapid genetic and phenotypic divergence is found in the salinity gradient of the Baltic Sea that has caused strong local adaptation (Serrão et al., 1996, 1999; Lago-Leston et al., 2010), contributing to a rapid speciation event in *Fucus vesiculosus* (Pereyra et al., 2009). Intraspecific divergence in physiological tolerance to emersion stressors (desiccation, freezing) between *F. vesiculosus* populations from intertidal and Baltic habitats have occurred over the brief evolutionary history of the Baltic Sea (Pearson et al., 2000; Lago-Leston et al., 2010).

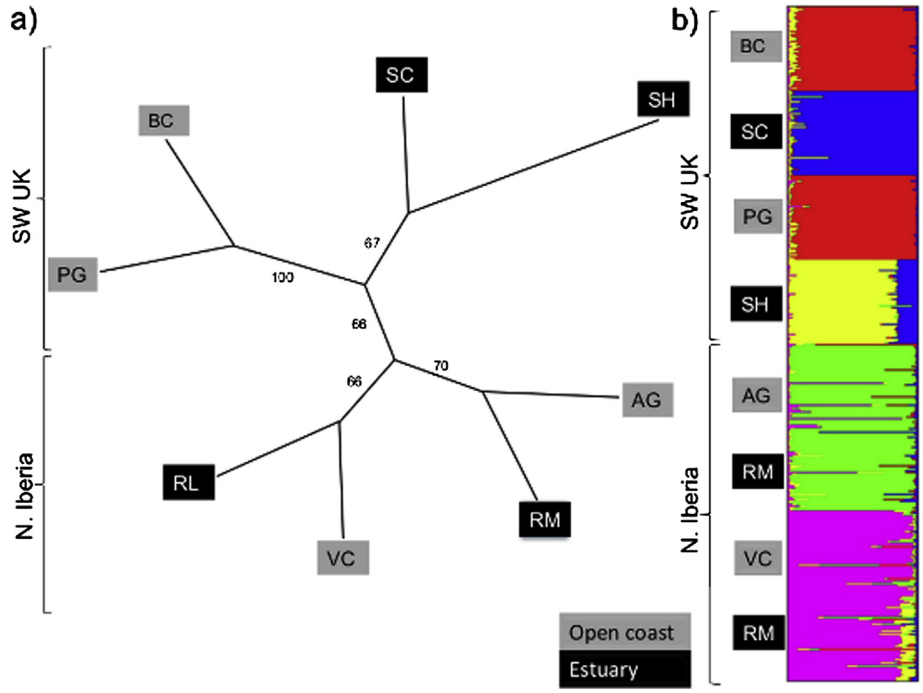

Fig. 5. Neighbour-Joining tree and histogram of STRUCTURE assignment of genotypes to clusters. Neighbour-Joining tree (a) inferred from Cavalli-Sforza and Edwards's pairwise distances; only bootstrap values higher than 50 are shown. Genetic structure (b) of populations defined by STRUCTURE revealed 5 groups (represented by distinct colours); each thin vertical line represents an individual and the proportion of each individual genotype assigned to each group is indicated by the colors. Codes correspond to regions and sites in Fig. 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In this study, all the estuarine populations experienced less pronounced air temperature fluctuations than open coast populations, implying a more predictable thermal and water-stress (desiccation) environment during emersion, although mean water and air temperature did not differ between habitats. Furthermore, at the regional scale, maximum temperatures during emersion were recorded at open coast sites, emphasizing the important role that habitat mosaics of environmental variation can play at local scales (Nicastro et al., 2010). Emersion times for *Fucus vesiculosus* varied strongly and consistently between habitats, independently of region (SW UK or N. Iberia); an increase of approx. 1.5 h for estuarine populations suggesting an upward vertical range shift relative to those on the open coast.

While both abiotic and biotic factors may play a role in facilitating vertical range extension inside estuaries, the less extreme and more predicable thermal environment may play a primary role. However, release from competitive interactions with higher shore congeners is also a plausible explanation. *Fucus spiralis* and *Fucus guiryi*, which are absent from the inner reaches of estuaries (Pérez-Ruzafa et al., 1993; Guiry and Guiry, 2012), coexist with *Fucus vesiculosus* on open coast rocky shores of the NE Atlantic and form belts in the intertidal zones above *F. vesiculosus* (Zardi et al., 2011a). While *F. vesiculosus* has lower resilience to emersion stress than *F. spiralis* and *F. guiryi*, indicating some physiological limitation on upper vertical limits (e.g. Dring and Brown, 1982; Beer and Kautsky, 1992), it can extend its vertical range higher on the shore when *F. spiralis* is removed (Hawkins and Hartnoll, 1985). This suggests that competitive release could contribute to the higher intertidal range of *F. vesiculosus* at estuarine sites.

Regardless of the underlying drivers, the increased average exposure times experienced by estuarine populations have been accompanied by increased resilience to extreme emersion stress that is maintained after acclimation to common conditions, indicating an adaptive response to differential selective pressures between habitats.

4.2. Neutral genetic divergence

It is of note that the physiological divergence between habitats, independently of region, was not matched by patterns of neutral genetic differentiation, suggesting that fundamentally different processes drive neutral divergence and phenotypic differentiation in this system. These results highlight that population differentiation estimated from neutral loci cannot be used to reveal the existence of sub-populations that might be differentiated for physiological traits that matter for fitness.

Regional patterns of neutral genetic differentiation result from the interplay between contemporary gene flow and historical processes. We found that the coastal sites in SW UK are highly connected and form a single genetic cluster, despite being >140 km apart, indicating present gene flow or insufficient divergence time for differentiation since the colonization of these sites. In contrast, N. Iberia coastal populations are more differentiated at smaller spatial scales (ca. 25 km), forming distinct clades with limited admixture. This pattern requires both a source of neutral divergence, such as sufficient time to accumulate differences and/or rapid drift in small populations, as well as low effective gene flow to maintain such differences. These conditions are found in the N. Iberia region, where the exposed open coast supports discontinuous *Fucus* patches, expected to limit stepping stone gene flow mediated by the typically low-dispersal fucoid gametes. Although *Fucus* species lack planktonic dispersal stages, they do have the potential to disperse by rafting of detached floating individuals (Muhlin et al., 2008), so the restricted dispersal between sites in this region may be due to oceanographic barriers preventing such transport (e.g. episodes of upwelling Alvarez et al., 2008a,b) limiting along-shore connectivity or low establishment success after dispersal. The latter hypothesis is plausible, given that in another *Fucus* species, such rafting gene flow appears to be only effective when colonizing new habitat, but does not become noticeable between even neighbouring populations due to priority colonization effects (Neiva et al., 2012).

Genetic divergence between the SW UK estuarine sites was lower than that between neighbouring habitats at each location. Such a pattern of relatedness cannot be explained by contemporary gene flow, and suggests that historical colonization processes might have played a role in shaping present day patterns of genetic structure. Although these data were not intended for use in reconstructing colonization/extinction history, our results raise the hypothesis that the similarity between populations within the same habitat type (estuarine or open coast) in SW UK could be the result of shared common ancestry (due to common colonization sources for populations of the same habitat) with rather limited recent gene flow. Under this scenario, present day divergence between the estuaries may in large part result from ongoing genetic drift in relatively small and isolated estuarine populations.

Genetic differentiation between coastal and estuarine habitats may also be determined by habitat continuity, an important determinant of genetic connectivity among populations (e.g. Alberto et al., 2010, for brown algae). In SW UK, neighbouring coastal and estuarine populations are separated by discontinuous habitat, mainly extensive mudflats that do not favour fucoid colonization. It is likely that habitat discontinuities, together with the limited dispersal of fucoid gametes and zygotes (e.g. Arrontes, 1993; Serrão et al., 1997), limit gene flow. In contrast, in N. Iberia, natural rocks, sea defences and marinas provide more substratum continuity between such habitats (pers. obs.) suggesting that the genetic connectivity between coastal and estuarine sites in this region occurs via “stepping stone” gene flow in the absence of distributional gaps from the coast to the inner estuaries.

Coastal topography and estuarine morphology can amplify the effects of habitat fragmentation, affecting circulation patterns, retention times and consequently species dispersal through rafting (Potter and Hyndes, 1999; Muhlin et al., 2008; Nicastro et al., 2008). The more sheltered nature of estuarine and coastal lagoon habitats leads to the prediction that the scale of gamete dispersal would be narrower, and thus population structure would be expected at a smaller spatial scale, but intrinsic characteristics of geomorphology of each estuary could influence circulation intensity and velocity (Day et al., 1989). For example, it is possible that more pronounced meander curvatures of English as opposed to the Iberian estuaries contribute to reduced dispersal efficiency and the discordant patterns of gene flow between habitats in N. Iberia and SW UK.

4.3. Conclusions

Estuaries are replicated natural environmental clines that provide the opportunity to investigate the roles that neutral and adaptive evolutionary processes play in population divergence between different habitats. In *Fucus vesiculosus*, variation in the degree of adaptive differentiation within different populations may be the key to understanding the changes in its distribution across spatial and temporal climatic variations (Nicastro et al., 2013). This study shows that a correlation between strong environment divergent selection and distinct physiological traits associated with emersion stress resilience is maintained independently of varying and discordant patterns of neutral genetic differentiation. Our findings highlight the need to consider both neutral genetic divergence and ecologically relevant adaptive traits for the appropriate identification of evolutionarily significant units of biodiversity and a functional definition of management and conservation efforts.

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Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.ecss.2013.08.016.

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