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Looking for diversity in all the right places? Genetic diversity is highest in peripheral populations of the reef-building polychaete *Sabellaria alveolata*

Flávia LD Nunes¹, François Rigal², Stanislas F. Dubois¹, Frédérique Viard^{3,4}

1. Ifremer, DYNECO, Laboratory of Coastal Benthic Ecology, F-29280, Plouzané, France

2. Université de Pau et des Pays de l'Adour

3. Sorbonne université, CNRS, Lab. AD2M, Station Biologique, Roscoff, France

4. present address : ISEM, Université de Montpellier, CNRS, EPHE, IRD, Montpellier, France

Abstract

Aim: To examine the consequences of changes in species distribution resulting from past climate change on the genetic diversity of the honeycomb worm, *Sabellaria alveolata*, a broadly distributed reef-builder that supports high biodiversity. To examine genetic connectivity within and among biogeographic provinces and to identify barriers to dispersal across the species range. To examine parallels between biogeography and phylogeography associated with this habitat-forming species in the Northeastern Atlantic.

Location: Boreal and Lusitanian Provinces in the Northeastern Atlantic

Taxon: The reef-building polychaete *Sabellaria alveolata*

Methods: Individuals of *S. alveolata* were sampled and the mitochondrial gene *cytochrome oxidase 1* sequenced from 20 locations spanning the Boreal and Lusitanian biogeographic provinces. Genetic diversity was assessed using six standard diversity indices. Differentiation

among populations was estimated using a hierarchical AMOVA and pairwise fixation indices (ϕ_{ST}). Tajima's D and Fu's F_s were calculated to test for deviations from neutral expectations and mismatch distributions were plotted for examining population expansion over all populations and over each geographic region examined.

Results: Genetic diversity in populations of *S. alveolata* was highest towards the edges of the current species range and lowest at its center. Pleistocene glacial cycles are likely responsible for the observed trend, causing extirpations of *S. alveolata* from the Bay of Biscay, but maintaining populations in glacial refugia in the English Channel and in North Africa. Northern populations host at least two sets of divergent haplotypes, indicating that two refugia possibly existed in the north. Within biogeographic regions, populations were overall well-connected, but strong genetic differentiation suggests that little exchange occurs between regions

Main conclusions: These two unexpected reservoirs of genetic diversity near either northern and southern range limits deserve greater attention as warming temperatures threaten trailing edge populations, while greater climatic variability threatens leading edge populations.

Introduction

Species distributions have been considerably modified by climate change over the course of geological time, culminating into present-day global biogeographic patterns (see (Spalding *et al.*, 2007; Briggs and Bowen, 2012) for marine systems). Ongoing climate change is expected to have similarly important effects on species distributions (Bellard *et al.*, 2012), with an increasing number of cases already documented (e.g., (Beaugrand *et al.*, 2014)). Species distributions and community structure are not the only aspects of biological diversity influenced by environmental change: intraspecific diversity is also affected, as the demographic changes that result from perturbations in climate leave their imprints on genetic diversity (Bowen *et al.*, 2016). Range shifts, expansions into new favorable habitat or local

extinctions can all affect neutral genetic variation, most commonly leading to loss in diversity (Pauls *et al.*, 2013). Likewise, selection under new environmental conditions and hitchhiking of linked loci can also reduce genetic variation, limiting the ability of a species or population to respond to a secondary stressor (Vogt *et al.*, 2010). In addition, environmental change may outpace evolutionary change, rendering some populations vulnerable. Rapid environmental change coupled with migration may also increase the inflow of maladapted genes selected under very different conditions into populations. At neutral loci, migration, from genetically differentiated sources, can also create, in climatic refugia, admixture due to secondary contact between previously isolated genetic lineages (refs. in (Maggs *et al.*, 2008). While life history traits, such as mode of reproduction or development, play an important role in dispersal ability and the capacity to evade climate change, extrinsic factors such as competition or physical barriers also limit where populations can thrive. The response of species to climate change therefore depends on the interaction of multiple factors, and is often difficult to predict.

In order to better apprehend how future climate change is likely to affect genetic diversity in marine populations, it is essential to first evaluate the patterns and processes that have shaped the current distribution of genetic diversity in the sea and to identify commonalities and idiosyncrasies among taxa. Pleistocene glacial-interglacial cycles have had profound effects on the European fauna and flora, strongly altering patterns of genetic diversity, such that expectations that diversity should be greatest at the central and lowest at marginal populations no longer hold. On land, the presence of extensive ice sheets during glacial periods restricted many species to southern refugia on the Iberian, Italian and Balkan peninsulas (Hewitt, 1999). Subsequent population expansion into ice-free areas during interglacials had long-lasting consequences on the genetic diversity of recolonized northern Europe. In the marine realm, glaciation also affected habitat availability. Eustatic sea-level drop by on average -90 m over the Pleistocene not only offset the current coastline offshore, but also altered the configuration and continuity of shallow marine habitats. For example, much of the English Channel was dry during Pleistocene glacials, and the Baltic Sea was covered by the Scandinavian ice sheet. Several marine glacial refugia are currently thought to have existed to the south of the ice sheets (Iberian Peninsula, Mediterranean Sea, Azores, Canary Islands and NW Africa). Many southern refugia now harbor unique genetic diversity not observed elsewhere that are now potentially under threat by climate change (Hampe and Petit, 2005), as trailing edges retract due to increasing temperatures in temperate and cold

seas (Poloczanska *et al.*, 2013; Pinsky *et al.*, 2020). Northern refugia in restricted areas that must have remained ice-free during glaciation have also been identified in parts of the English Channel, Ireland, Iceland and northern Norway (for a summary, see (Maggs *et al.*, 2008; Jenkins *et al.*, 2018). These northern refugia host pockets of high genetic diversity and were likely source populations for recolonization of glaciated coastal areas.

Among marine invertebrates, a number of different scenarios of post-glacial colonization have been described (Wares and Cunningham, 2001; Maggs *et al.*, 2008; Jenkins *et al.*, 2018), demonstrating a variety of possible responses to Pleistocene climate change. Here we propose to study the response of an important foundation species to past climate change, the common and broadly distributed reef-building polychaete *Sabellaria alveolata*.

The honeycomb worm *S. alveolata* is an ecosystem engineer that sustains high biodiversity (Dubois *et al.*, 2002; Jones *et al.*, 2018) and builds the largest biogenic reefs in Europe (Noernberg *et al.*, 2010). It is distributed throughout much of the intertidal rocky coast of temperate Europe, from northern Ireland to northern Mauritania (Curd *et al.*, 2020). Biological traits can vary over long time scales, but currently, *S. alveolata* is gonochoric, reaching maturity within its first year, and exhibiting one to two major reproductive peaks per year depending on its distribution, followed by trickle spawning throughout the remainder of the year (Gruet and Lassus, 1983). Mean planktonic lifetime has been estimated at 4-10 weeks based on field observations (Dubois *et al.*, 2007) although under experimental conditions, larvae appeared to be competent only by 6 weeks (Wilson, 1968a) and can be kept alive in controlled conditions upwards of 14 weeks (our unpublished results). Recruitment takes place preferentially on existing conspecific reefs (Wilson, 1968b), although settlement on other substrates such as sand, rock or even artificial hard substrate such as seawalls is also possible (Pawlik, 1988; Firth *et al.*, 2015). The species is rather tolerant to heat stress, being able to acclimatize to sustained high temperatures by modulating its cellular membrane lipids, although this ability appears to be limited to temperatures below 25C (Muir *et al.*, 2016). However, it appears to be sensitive to cold temperatures, with local population collapse being observed during severe winters (Firth *et al.*, 2015).

The objective of this study was to address the following questions:

1. Did Pleistocene climate change affect genetic diversity of *S. alveolata*?
2. Did *S. alveolata* persist in glacial refugia?
3. What is the genetic structure of this taxon in relation to the biogeographic provinces in which it is encountered?

We conclude with a discussion on how the answers to these questions can better inform future conservation measures for this important reef-builder.

Methods

1. Sampling

Individuals of *S. alveolata* were sampled between 2003 and 2005 from 20 sites, encompassing two biogeographic provinces (Boreal and Lusitanian Provinces, *sensu* (Golikov *et al.*, 1990)), both representing a substantial part of the present-day distribution range. Sampling included nine localities in the Boreal Province (five on the Irish Sea coast of England and four on the French coast of the English Channel), one site on the Iroise Sea in France (located in the transition zone between Boreal and Lusitanian provinces), and nine localities in the Lusitanian Province (eight from the Bay of Biscay in France and Spain and one site on Atlantic coast of Morocco). One hundred individual worms from several veneers and reef blocks were collected at each site. Worms were delicately removed from their tubes and kept in 95% ethanol until DNA extraction.

2. Molecular Analysis

2.1 DNA Extraction, amplification and Sanger sequencing

DNA was extracted from the cephalic region of 32 individuals from each sampled locality, using the Nucleospin 96 Tissue kit (Macherey-Nagel), and following manufacturer protocol. The mitochondrial *cox-*

1 gene was amplified by polymerase chain reaction using universal primers (Folmer *et al.*, 1994). The PCR reaction had a total volume of 25 μ l and consisted of 2.5 μ l of 10x buffer containing MgCl₂, 2 μ l of 10 mM dNTPs, 0.5 μ l of BSA at 1 mg/ml, 1 μ M of each of 10 mM primers (LCO-1490 and HCO-2198), 0.1 μ l of Thermoprime Hi Fidelity Taq polymerase (ABgene) and 2 μ l of template DNA (1:10 to 1:20 dilution of the DNA stock solution). PCR reactions were conducted in a MJ Research PTC-200 Peltier thermal cycler. The samples underwent an initial denaturation step at 94°C for 3 min, followed by 5 cycles with denaturation at 94°C for 55 sec, annealing at 45°C to 49°C (+1°C at each cycle) for 45 sec and extension at 72 ° C for 1 min 10 sec, and an additional 35 cycles as before, but with an annealing temperature at 50°C. The results of the amplification were visualized under UV light after electrophoresis on a 1% agarose gel (ultra-pure DNA-grade agarose, Eurogenetec) containing ethidium bromide.

The PCR products (680 base pairs) were purified on a Multiscreen membrane plate (Millipore) to remove excess primers and nucleotides. Cycle sequencing was carried out with Big Dye Terminator v3.1 chemistry on a Gene AMP thermocycler (Applied Biosystems). The reaction solution had a total volume of 5 μ l and was composed of 0.5 μ l of Big Dye, 0.75 μ l of 5x Buffer, 0.5 μ l of 10 μ M forward or reverse primers, 1.25 μ l of purified DNA and 1.75 μ l of ultrapure water. The cycle sequencing reaction was purified and run on an ABI prism 3130XL Genetic Analyzer 16 capillary automatic sequencer.

Approximately half of the PCR products were sequenced in both reading directions, while the remainder was sequenced in only one direction. Sequences with low signal:noise were systematically re-sequenced, as were haplotypes with singletons. Only individuals having common haplotypes were sequenced in just one direction.

3. Data analysis

Sequence chromatograms were visually inspected for accuracy of base calls using the ABI Chromas software. Bioedit was used for sequence alignment over the course of data acquisition. Once the complete dataset was finalized, sequences were aligned with ClustalX, using the default parameters (Thompson *et*

al., 1997), and trimmed to a final length of 537 base pairs to ensure high-quality sequence over all individuals.

3.1. Genetic diversity

Geographic patterns of genetic diversity were examined by calculating haplotype frequencies, the number of haplotypes (N_h), haplotype richness (H_r), nucleotide diversity (π), the number of private alleles and the number of segregating sites (S) for each sampling site. Indices were calculated using DNAsp v5 (Librado and Rozas, 2009) and Arlequin v3.5 (Excoffier and Lischer, 2010). For haplotype richness (H_r), the rarefaction procedure described in (El Mousadik and Petit, 1996) was implemented in R using the vegan package (Oksanen *et al.*, 2009). The non-parametric Mann-Whitney U test was used to evaluate statistical significance in differences in the average values of each genetic diversity index among *a priori* defined regions (Irish Sea, English Channel and Bay of Biscay).

3.2 Demography and admixture inferences

Assuming neutrality, in order to detect deviations from mutation-drift equilibrium, Tajima's D (Tajima, 1989) and Fu's F_s (Fu, 1997) were calculated in Arlequin v3.5. Fu's F_s is particularly relevant for datasets characterized by a large number of private haplotypes, as in this study, and has greater power to detect recent population expansion. The distribution of pairwise differences between haplotypes ("match-mismatch" distribution), was applied across the whole dataset, by region and within each population using DNAsp. "Match-mismatch" distributions are an indicator of demographic processes such as population expansion or admixture between groups of genetically differentiated individuals (Rogers and Harpending, 1992). If a population is composed of haplotypes that have recently evolved from the same common ancestor, divergence will be small among haplotypes and the number of differences between two haplotypes taken at random will be low. The "match-mismatch" distribution would then have a single peak with low mean value. On the other hand, in a population resulting from admixture of individuals from two divergent populations, the number of differences between two sequences taken at

random in the population can be low if the haplotypes drawn are from the same source population or high if the haplotypes are drawn from two divergent groups (admixture). In the case of admixture, the “match-mismatch” distribution presents two peaks.

3.3 Geographical patterns of haplotype diversity

The distribution of haplotypes among the studied populations provides an overview of the levels of genetic similarity among populations. Haplotype frequencies and shared haplotypes were calculated in Arlequin. An analysis of the genetic structure among populations was then performed through an analysis of molecular variance (AMOVA; (Excoffier *et al.*, 1992), and by estimating pairwise fixation indices (ϕ_{ST}), which take into account haplotype frequencies as well as the number of mutations between haplotypes. Statistical significance was evaluated by a permutational test (haplotype resampling across populations) in order to test the null hypothesis of genetic identity among populations ($\Phi_{ST} = 0$). As localities belong to distinct regions and biogeographic provinces, a hierarchical AMOVA was carried out to test for a “Region” effect, by computing Φ_{CT} , measuring the genetic variance between groups, as compared to the overall genetic variance in the dataset.

A Mantel test (Mantel, 1967) was used to compare a matrix of ϕ_{ST} (linearized by $\phi_{ST}/(1-\phi_{ST})$) and a matrix of geographical distances computed between each population pair, to test the existence of a correlation between the geographical distribution and genetic distances between populations (Rousset, 1997). The matrix of ϕ_{ST} was obtained with Arlequin, geographical distances were measured using Encarta software. The geographic distance matrix was designed from the coastal distances between populations. This test was performed using Genepop v3.4 (Raymond and Rousset, 1995). This program performs a non-parametric matrix comparison test according to Mantel (1967). The value of an association parameter Z (the Mantel coefficient) between the two matrices was calculated from the real data, then compared to a series of pseudoreplicates obtained by random permutation of the order of the populations (10000 permutations) in one of the two distance matrices.

3.5 Network analysis

A haplotype network was used to visualize the evolutionary relationships (genealogies) among haplotypes. Minimum spanning, median joining and TCS (statistical parsimony) algorithms were tested using PopART v1.7 (Leigh and Bryant, 2015). Because of the high number of unique haplotypes present in the dataset, all algorithms showed reticulation in the network. The TCS algorithm was selected for having the lowest level of reticulation. Nevertheless, the TCS haplotype network calculated with the full dataset was highly complex, with many interconnections. In order to simplify the network such that it could be better interpreted, the dataset was split into three groups: Irish Sea, English Channel+Iroise Sea, Bay of Biscay+Moroccan Coast. In all 3 networks, shared haplotypes showed the overall haplotype frequencies over all sampled regions, to facilitate comparisons.

Results

1. Haplotype distribution and genealogy

A total of 176 haplotypes were observed among the 583 studied individuals (Genbank accession numbers MT955994-MT956576). Of these, only 21 haplotypes were shared by at least two of the 20 populations, with three being common and representing more than half of the individuals (350 or 60%) (Figure 1 and 2). The most common haplotype was observed in all populations (H14; n=247). The second-most abundant haplotype was observed only in the Bay of Biscay (H138; n=69). The third most common haplotype (H26; n=39) occurred at high frequencies in two populations of the Irish Sea, and was observed at minor frequencies in one population of both the English Channel and the Bay of Biscay. The remaining haplotypes shared among populations occurred at less than 8% frequency per population. A total of 153 haplotypes (87%) were private, i.e. present in a single population. Only 7 private haplotypes were observed more than once. The number of private haplotypes was greatest in the Irish Sea and English Channel, with the highest number occurring in Saint-Anne (English Channel; n=26). Populations in the Iroise Sea and Bay of Biscay had generally fewer private haplotypes (<6 whatever the population). The reef located on the Moroccan coast had an intermediate number of 8 private haplotypes. The geographical distribution of haplotypes (by population) is shown in Figure 1.

The haplotype network built with the TCS method for all haplotypes, while being less reticulated than those constructed with the minimum spanning and median joining algorithms had numerous loops and multiple connections among nodes. In order to better interpret the relationships among haplotypes, 3 sub-networks were constructed: Irish Sea, English Channel + Iroise Sea, Bay of Bisca + North Africa (Figure 2). All three networks had a star shape, with the most common haplotype H14 located at the center. The two following most common haplotypes were separated by a single mutational step from H14: H26 was common in both the Irish and Iroise Seas, and H138 was common but exclusive to the Bay of Biscay. All regions had haplotypes present in the "first crown" of the network (i.e., one mutational step from the central haplotype), as well as within the second ring (2 mutations). From this central network, connections became more complex as haplotypes were more divergent (up to 11 mutations). Both the Irish Sea and English Channel had multiple divergent haplotypes (>4 mutations from the central haplotype H14) that clustered together. One divergent haplotype was observed in the Bay of Biscay and two on the North African coast (Figure 2).

2. Population genetic diversity

At the population level, five indices of genetic diversity all showed greater levels of diversity in the Irish Sea, English Channel and North Africa than populations of the Iroise Sea and Bay of Biscay (Figure 1 and 3). Rarefied haplotype richness ranged from 8.2 to 16.0 in the Irish Sea, from 11.9 to 17.5 in the English Channel, and was 13 in North Africa, while in the Bay of Biscay and Iroise Sea it ranged from 2.3 to 6.8 (Figure 1). Mann-Whitney U for H_{rar} values confirm significantly lower values in the Bay of Biscay compared to Irish Sea and English Channel ($p=0.001$ and $p=0.003$, respectively). Likewise, the number of haplotypes and nucleotide diversity were consistently greater in the Irish Sea, English Channel and North Africa, and lower in the Bay of Biscay and Iroise Sea. Two notable exceptions were the populations of Shell Island and Aberporth in the Irish Sea, which had consistently lower values of genetic diversity for the five indices (Figure 3). The number of private alleles was strikingly different among geographic regions, being as large as 23 in Sainte-Anne (English Channel), but no larger than 6 in the populations of the Bay of Biscay. Similarly, the number of segregating sites was largest at Dubmill, the northernmost population of *S. alveolata* ($s = 47$), while in the Bay of Biscay, s ranged from 2 to 23 (Figure 3)

3. Population genetic structure

Analysis of molecular variance (AMOVA) indicated significant population genetic structure at all hierarchical levels (Figure 4). Over all populations, the fixation index was strong and significant ($\Phi_{ST} = 0.101$, $p < 0.0001$). Variation among regions (Irish Sea, English Channel and Bay of Biscay) explained 6.57% of the variance, and Φ_{CT} was significant ($\Phi_{CT} = 0.067$, $p < 0.0001$). Differentiation was also significant among populations within groups (i.e., regions, $\Phi_{SC} = 0.037$, $p < 0.0001$), suggesting genetic drift and/or limited connectivity among populations, even at the regional level.

Pairwise fixation indices (ϕ_{ST}) are given in Figure 4. Genetic differentiation within the Irish Sea was significant for 6 out of 10 pairwise comparisons. None of the pairwise ϕ_{ST} values were significant within the English Channel. In the Bay of Biscay, the majority of comparisons were also not significant with the exception of comparisons that included Arcachon and Galicia (although differentiation was not significant between these two populations). In agreement with the hierarchical AMOVA, at the inter-regional scale, differentiation was strong and significant for nearly all comparisons with the Bay of Biscay. However, ϕ_{ST} between the Irish Sea and English Channel populations were lower and only significant for 35% of the comparisons.

The Mantel test performed on all populations except Morocco ($n=19$) showed that isolation by distance was significant ($r = 0.416$, $p = 0.0046$). On the other hand, Mantel tests were not significant within the regions (Irish Sea $r = 0.256$, $P = 0.962$, Channel $r = 0.848$, $P = 0.120$, Bay of Biscay $r = -0.070$, $P = 0.500$). These results illustrate a strong isolation by the distance at the regional level.

4. Demographic changes

Tajima D and Fu's F_s were negative and significant for nearly all populations in the Irish Sea, English Channel and North African coast, with the exception of Aberporth (Irish Sea), indicating population expansion (under the assumption of neutrality)(Figure 4.C). In contrast, these two indices were both

significant for only 3 out of 9 populations in the Bay of Biscay and the Iroise Sea, with two other populations having one or the other index being significant (Figure 3), suggesting mutation-drift equilibrium. Most of these results are supported by match-mismatch analyses.

Match-mismatch distributions were constructed for all hierarchical levels of the dataset. The results are illustrated by 4 curves: (i) over all populations, (ii) for the Irish Sea, (iii) for the English Channel and (iv) for the Bay of Biscay (Figure 5). The mismatch curve over all populations was not significantly different from the expected distribution at the mutation-drift equilibrium, suggesting a stable "global population" (Figure 5 A). The profile expected under mutation-drift equilibrium is found in all the populations of the Bay of Biscay individually and the Iroise Sea (not shown) as well as at the regional level for the Bay of Biscay (Figure 5 D). Conversely, the curves obtained at the regional level for the Irish Sea and the English Channel present profiles that indicate deviations from the neutral expectation (Figure 5 B and C). Both the Irish Sea and English Channel distributions have more than one peak, indicating groups of divergent haplotypes. The populations of Dubmill, Nashpoint, Champeaux and Sainte-Anne have an even more complex distribution, reflecting the presence of several groups of divergent haplotypes ranging from a difference of 5 to 10 mutations on average (not shown). Curves with two or more peaks are expected to result from admixture between divergent lineages.

Discussion

Phylogeography considers the spatial distribution of alleles within a species in order to evaluate its recent evolutionary history. For the honeycomb worm, *S. alveolata*, patterns of genetic diversity and differentiation reveal how past climate change has influenced the distribution of the species, and how current conditions affect connectivity.

Contrasting patterns of genetic diversity across seas, in the context of post-glacial colonization

Our results show that genetic diversity is highest in the Irish Sea and the English Channel, two regions that are close to the present-day northern range limit of the species (Scotland/Ireland), while it is

reduced and significantly lower in the Bay of Biscay, the current central core of the species range. These results may appear counterintuitive at first glance, but they are coherent with patterns of post-glacial colonization that have been observed for other marine invertebrates (Maggs *et al.*, 2008). During Pleistocene glacial cycles, ice sheets extended across much of Europe, but a few areas are thought to have remained ice-free. While most of the English Channel was above the sea level line, it is thought that deep sectors, such as the Hurd Deep, remained ice-free and served as refugia for marine organisms (Provan *et al.*, 2005; Hoarau *et al.*, 2007). This scenario is coherent with patterns of genetic diversity for *S. alveolata*. The Irish Sea and English Channel have higher levels of haplotype diversity and nucleotide diversity than other regions. Furthermore, these two regions have a greater number of private haplotypes.

Interestingly, both regions host at least one cluster of divergent haplotypes, as seen in the haplotype network and in the mismatch distributions. The Irish Sea and English Channel haplotype networks have a topology that resembles a contact zone between multiple refugia, but the origin of the divergent haplotypes remains unknown. These divergent haplotypes were likely maintained and/or evolved in separate refugia during a, or multiple, previous glacial periods, and migrated to the Irish Sea and English Channel during a subsequent interglacial. Further studies are needed to identify the source of these haplotypes, but here we speculate that Ireland could potentially have been a glacial refugium for *S. alveolata*, as has been proposed for the red seaweed *Palmaria palmata* (Provan *et al.*, 2005), for the brown seaweed *Fucus serratus* (Hoarau *et al.*, 2007) and the marine gastropod *Littorina saxatilis* (Panova *et al.*, 2011), all of which commonly co-occur with *S. alveolata* on rocky shores.

The Bay of Biscay mostly harbors haplotypes that have only a few mutational differences from two common haplotypes. This haplotype network topology, coupled with the observation that genetic diversity is moderate in this region, suggests that the Bay of Biscay has been colonized by *S. alveolata* more recently than other geographic regions, or that colonization occurred with founder events. It is however interesting to note that the highest levels of genetic diversity are observed in Yeu Island, La Fontaine aux Bretons and Tranche-sur-Mer, three central populations within the Bay of Biscay. At the regional level, genetic diversity does appear to be greatest in central and lower in marginal populations, as is usually expected.

A single site was examined from the North African coast. The population from El Jadida, Morocco, showed strong differentiation with respect to most sites in the Bay of Biscay, but pairwise ϕ_{ST} were weak and several were not significant with respect to populations in the Irish Sea and English Channel. Given the large distances that separate these sites, it appears likely that weak fixation indices are the result of shared ancestral polymorphism. Common haplotypes may have been maintained in separate refugia, leading to the apparent genetic similarity at opposite range edges. Genetic diversity in this population in Morocco is also relatively high, and presents a profile of demographic expansion (high H_e , with negative and significant values of Tajima's D and F_s), which could indicate the existence of another refugium in the southern part of the distribution, which unfortunately remained unsampled in our study. We also lack populations along the Iberian Peninsula to get a comprehensive picture of the patterns in the southern part of the Lusitanian distribution. The southern range of *S. alveolata* remains an interesting area for further research. Additional glacial refugia may be found on the coasts of the Iberian Peninsula and North Africa, as has been observed for (refs). High genetic diversity in the Iberian coast has been observed for the red alga *Chondrus crispus* (Provan and Maggs, 2012). However, the authors argue that due to restricted connectivity among Iberian and northern populations, the unique genetic diversity observed in the rear edge of *C. crispus* is threatened by increasing global temperatures. Zones of upwelling in the Iberian and North African coasts have also been shown to harbor higher genetic diversity in the macroalga *Fucus guiryi*, a phenomenon that has been attributed to more stable demography in areas where temperatures remain cool (Lourenço *et al.*, 2016). It would be interesting to examine whether this also holds true for *S. alveolata*. Future work that examines populations in the Iberian Peninsula and other Northern African localities are therefore essential for understanding the spatial patterns of genetic diversity in *S. alveolata* throughout its range, and to evaluate the potential impacts of past and future climate change in the trailing edge of the distribution.

Congruences between biogeography and phylogeography

Analysis of molecular variance revealed significant genetic differentiation among regions ($\Phi_{CT} = 0.066$, $p < 10^{-5}$), particularly between the Bay of Biscay and the other two studied regions to the north. The

observation that the second most common haplotype has arisen in the Bay of Biscay and is not found in any other region further supports the interpretation of limited migration out of the Bay of Biscay. Regardless of the source of the historical colonization of the Bay of Biscay, this area ceased to exchange or exchanges very little with the two regions to the north. Conversely, genetic differentiation between the Irish Sea and English Channel was moderate, with significant ϕ_{ST} values being observed only with respect to the Shell Island and the Lannion populations. For *S. alveolata*, the absence of a strong barrier to gene flow between the Irish Sea and the English Channel may in part be explained by the similarities in high genetic diversity and population expansion profiles observed in both regions. Furthermore, it is possible that both regions were recolonized from the same glacial refugium (such as the Hurd Deep). Connectivity between the Irish Sea and English Channel is also consistent with the long pelagic larval duration of *S. alveolata*.

Altogether *S. alveolata* provides an interesting case for considering the congruence between biogeography and phylogeography that has been recently shown to occur in various taxonomic groups throughout the globe (Bowen *et al.*, 2016). Biogeographic provinces are defined based on species distributions, their range limits and levels of endemism (species that are only found within the province). In addition, characteristics of a biome (the physical and chemical properties of a region) can also help to identify biogeographic provinces. The delimitation between the Boreal and Lusitanian Provinces remains contentious with respect to their boundary, and whether it should be placed at the entrance, middle or outlet of the English Channel, or dividing the southern and northern coasts of the English Channel (summarized in (Dinter, 2001)). Based on an in-depth review of species distributions and biome characteristics, (Dinter, 2001) proposed five biogeographic regions that encompass the Irish Sea, English Channel and Bay of Biscay. However, these regions have not been universally adopted in the biogeographic literature (see (Spalding *et al.*, 2007; Briggs and Bowen, 2012)). Phylogeography of *S. alveolata* shows intraspecific homogeneity throughout the Irish Sea+English Channel and within the Bay of Biscay, but marked differences between them. If biogeography and phylogeography tend to indeed be congruent, then our data would support a delimitation of the Boreal and Lusitanian provinces at the western entrance of the English Channel (in other words, at the Iroise Sea). While similar patterns in phylogeography have been observed for other polychaetes and molluscs whose distribution spans the Iroise Sea (Luttikhuisen *et al.*, 2003; Jolly *et al.*, 2005, 2006), several other marine invertebrate taxa do

not show genetic differentiation across this putative biogeographic barrier (Roman and Palumbi, 2004; Palero *et al.*, 2008; Couceiro *et al.*, 2012; Halanych *et al.*, 2013; Taboada and Pérez-Portela, 2016). Biogeographic delimitation is rendered difficult in this part of the North Atlantic by the high heterogeneity of environmental parameters over relatively short spatial scales coupled with a complex glacial history, both of which may have varying effects in different taxonomic groups, leading to divergent patterns in species distributions over geological time. Although there does not appear to be strict consensus with respect to the position of phylogeographic boundaries among benthic marine invertebrates in the eastern North Atlantic, there does appear to be a general pattern of distinctiveness between the Bay of Biscay and the areas north of the English Channel (Maggs *et al.*, 2008), suggesting that broadly speaking, phylogeography does appear to parallel biogeography among eastern North Atlantic marine species.

The interaction between bottleneck events and hydrodynamic features modulate patterns within regions

We previously showed that the present-day connectivity appears limited between biogeographic regions. Analysis of molecular variance also showed significant differentiation among populations within regions ($\Phi_{sc} = 0.037$, $p < 10^{-5}$). However, much of this within-regional differentiation can be attributed to the populations within the Irish Sea, where 70% of pairwise comparisons showed significant values of ϕ_{ST} . In particular, the populations of Shell Island and Aberporth are differentiated from all other populations (except each other, and both are in Cardigan Bay) and Nash Point is differentiated from all other populations except Duckpool (both in the Bristol Channel). This genetic differentiation might be due to limited exchange but also to the influence of genetic drift, notably through bottleneck events. The populations of Shell Island and Aberporth indeed both have a smaller number of haplotypes and lower values of haplotype and nucleotide diversity than the other three Irish Sea sites. Lower values of genetic diversity in Cardigan Bay are consistent with historical records that show that the cold winter of 1962/1963 led to the mortality and long-term absence of *S. alveolata* from the coast of North Wales (Firth *et al.*, 2015). Cold climate conditions that lasted nearly 20 years and prevented the re-establishment of *S. alveolata* reefs in this area could explain the observed reductions in genetic diversity. Shell Island and Aberporth have likely only recently been colonized from adjacent reefs, and harbor only a subset of the regional genetic diversity. The population of Nash Point in the Bristol Channel is also significantly

differentiated from all other populations to the north. Populations in the Bristol Channel of the common cockle *Cerastoderma edule* are also genetically differentiated from a population only 50 km away just outside of the channel (Dale) (Coscia *et al.*, 2013). Hydrodynamic and particle transport models for *C. edule* across the Celtic and Irish Seas indicate that tidally-driven flows in the Bristol Channel have a net transport up the estuary (flood-dominated flows) that lead to high local retention (Coscia *et al.*, 2013). In sum, the high genetic structure of *S. alveolata* within the Irish Sea is likely the result of a combination of factors, including complex circulation patterns, but also because of demographic instability near the current range edge that has led to local bottlenecks and loss of genetic diversity.

Genetic structure within the other two studied regions had a much different pattern. Within the English Channel, pairwise ϕ_{ST} values were non-significant for all comparisons among populations. Three of the four sampled populations were all within the Bay of Mont St Michel, with distances ranging from 25 to 50 km. Larval exchange is probably maintained throughout this bay, leading to the homogenization of haplotypes within the bay, in agreement with larval dispersal models for *S. alveolata* (Ayata *et al.*, 2009). Surprisingly, genetic differentiation was not observed with respect to Lannion, a site which is ~200 km away. Connectivity with this site is likely maintained across multiple generations or through intermediate stepping-stone reefs not sampled in this study.

Genetic differentiation was also generally not significant within the Bay of Biscay. The Bay of Biscay has many *S. alveolata* reefs, at short distances from one another. This likely facilitates larval exchange and maintains genetic connectivity. The only exceptions were the Arcachon and Galicia populations in the southern part of the Bay of Biscay, for which 10 out of 15 pairwise comparisons had a significant ϕ_{ST} . Arcachon and Galicia are the only sites sampled in our study to the south of the Gironde River outflow. The Gironde River has a strong outflow, characterized by low salinity surface waters in winter and spring that generally flow northward and along shore in winter, and offshore in spring (Lazure and Jegou, 1998). The Gironde outflow could therefore be a potential barrier to dispersal, either due to low salinity or because of its impact on local circulation. As adults, sabellarids are known for being tolerant to brackish waters (Mauro, 1977; Lana and Gruet, 1989) (Mauro, 1977; Lana and Gruet, 1989), but tolerance thresholds for salinity have not yet been quantified for *S. alveolata* larvae, which could potentially be more vulnerable in this life stage. Low salinity, northward flow, or geographic distance from other reefs

(Arcachon is located approximately 100 km south of the mouth of the Gironde), could contribute to the relative isolation of this site. It is interesting to note that Arcachon and Galicia are not differentiated with respect to each other, indicating some level of connectivity between these two southern Bay of Biscay sites. Additional sampling in the north of Spain could help clarify whether Arcachon is indeed an isolated reef, or if exchange occurs with other sites south of the Gironde and along the northern coast of Spain.

Conclusions

The objective of this study was to address whether and how Pleistocene climate change affected genetic diversity of *S. alveolata*, to identify glacial refugia for the species, and to relate the genetic structure of this taxon to currently recognized biogeographic provinces. Our observations show that Pleistocene climate change had important and long-lasting effects on the genetic structure and diversity of *S. alveolata*.

Glaciation led to extirpation of populations in the Bay of Biscay with subsequent post-glacial colonization which has led to reduced genetic diversity throughout the region. The species persisted in at least one glacial refugium in the English Channel or Irish Sea. Given that a glacial refugium has been identified in the Hurd Deep of the English Channel, it seems plausible that this served as a refugium for *S. alveolata* as well. Divergent haplotypes in populations of the Irish Sea and the English Channel also suggest that a second glacial refugium may also have existed in the northern part of the range. Here we speculate that this refugium could have been in Ireland, as has been observed for intertidal marine macroalgae. High genetic diversity observed in the North African population also suggests that southern glacial refugia existed for *S. alveolata*, but further studies are required to confirm precisely where and how many southern refugia existed. Our results warn against over-simplifications of central-edge predictions in genetic diversity, as here we find the opposite trend: high diversity at the edges and low diversity in the central core. Finally, our results lend support to the prediction that phylogeography is congruent with biogeography, as Boreal and Lusitanian biogeographic provinces are each composed of populations that seem to maintain connectivity and have similar levels of genetic diversity. Despite some idiosyncrasies, glacial-interglacial cycles have left an important imprint within and among species in the eastern North Atlantic.

Population genetics and conservation

Our results have a number of implications for conservation of this important ecosystem engineer. This study highlights the Irish Sea and English Channel as two important reservoirs of genetic diversity for the species. The Irish Sea encompasses the northern range edge of *S. alveolata*, an area that could be more prone to demographic instability and population collapse. Although climate change scenarios predict general warming, they also predict more variable and extreme weather events that could be unfavorable to *S. alveolata*. Cold winters have already led to decadal scale local extinctions of *S. alveolata*, resulting in loss of genetic diversity locally. In order to prevent widespread loss of the species in this potentially more unstable part of the range, monitoring and preservation of populations in the British Isles should be prioritized. The English Channel is also an important reservoir of genetic diversity, with seemingly well-connected populations at the regional level. However, the lack of connectivity with southern parts of the range means that English Channel populations are unlikely to be a source of larvae to other regions. Furthermore, due to their relative isolation with the rest of the range, the English Channel populations should be managed as a self-sustaining unit. Populations of *S. alveolata* in the Bay of Biscay populations appear to be numerous (sampling was conducted on 8 populations), and our results suggest they are well-connected among each other. This likely renders this central region of the distribution more stable. However, the Bay of Biscay has a genetic background that is distinct from the Irish Sea and English Channel, suggesting a different evolutionary history. In order to preserve the evolutionary potential of *S. alveolata*, populations in Bay of Biscay should be considered as an Evolutionarily Distinct Unit (sensu (Coates *et al.*, 2018) in conservation programmes. In addition, future research that focuses on populations to the south of the Gironde River is required to determine whether these populations are isolated. Finally our work addresses only a part of the species distribution. *S. alveolata* is present and abundant in certain parts of the Iberian and North African coasts, but little is presently known about these populations. Future work that includes denser sampling along the southern part of the range could point to other important sources of genetic diversity and evolutionary distinctiveness in the trailing edge of the distribution.

Figures

Figure 1. Map of the twenty sampling locations of *Sabellaria alveolata* collected for this study. The size of the violet circle at each location corresponds to haplotype richness at the site, following rarefaction to account for unequal sample sizes. Haplotype frequencies are shown as pie charts connected to each site by a black line, and numbered 1 to 20 as in Figure 4. Alleles that were rare (present in two populations, and at low frequencies) or private (present in only one population) were grouped as two respective categories.

Figure 2. Haplotype networks for A. Irish Sea, B. English Channel and Iroise Sea, C. Bay of Biscay and North Africa. Haplotypes common to more than one region are shown with the frequencies observed over the whole dataset (all regions). The positions of shared haplotypes were kept in the same positions to facilitate comparisons.

Figure 3. Molecular diversity indices for each population, including number of haplotypes, nucleotide diversity, number of private alleles and number of segregating sites. Each population is colored according to the region in which it was sampled (see legend).

Figure 4. Genetic differentiation among populations. A. Pairwise ϕ_{ST} calculated for each pair of populations. The black triangles indicate comparisons within the Irish Sea, English Channel and Bay of Biscay regions, from top to bottom. Cell color corresponds to significance values, as shown in the legend. B. Analysis of Molecular Variance (AMOVA) indicates that differentiation is significant at all hierarchical levels of the dataset: among populations, among regions, and among populations within regions. C. Values of Tajima's D and Fu's Fs for each population sampled.

Figure 5. Mismatch distribution calculated over A. All populations combined, B. Populations located in the Irish Sea; C. populations located in the English Channel and D. populations located in the Bay of Biscay. Observed values of the mismatch distribution are shown as circles, and expected values are shown as a continuous line. Significant differences between expected and observed values occur for the Irish Sea and English Channel, but are not significant for the Bay of Biscay and over all populations.

Data Availability

Newly generated mtDNA sequences have been deposited in GenBank (Accession Numbers: MT955994-MT956576).

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Figure 1

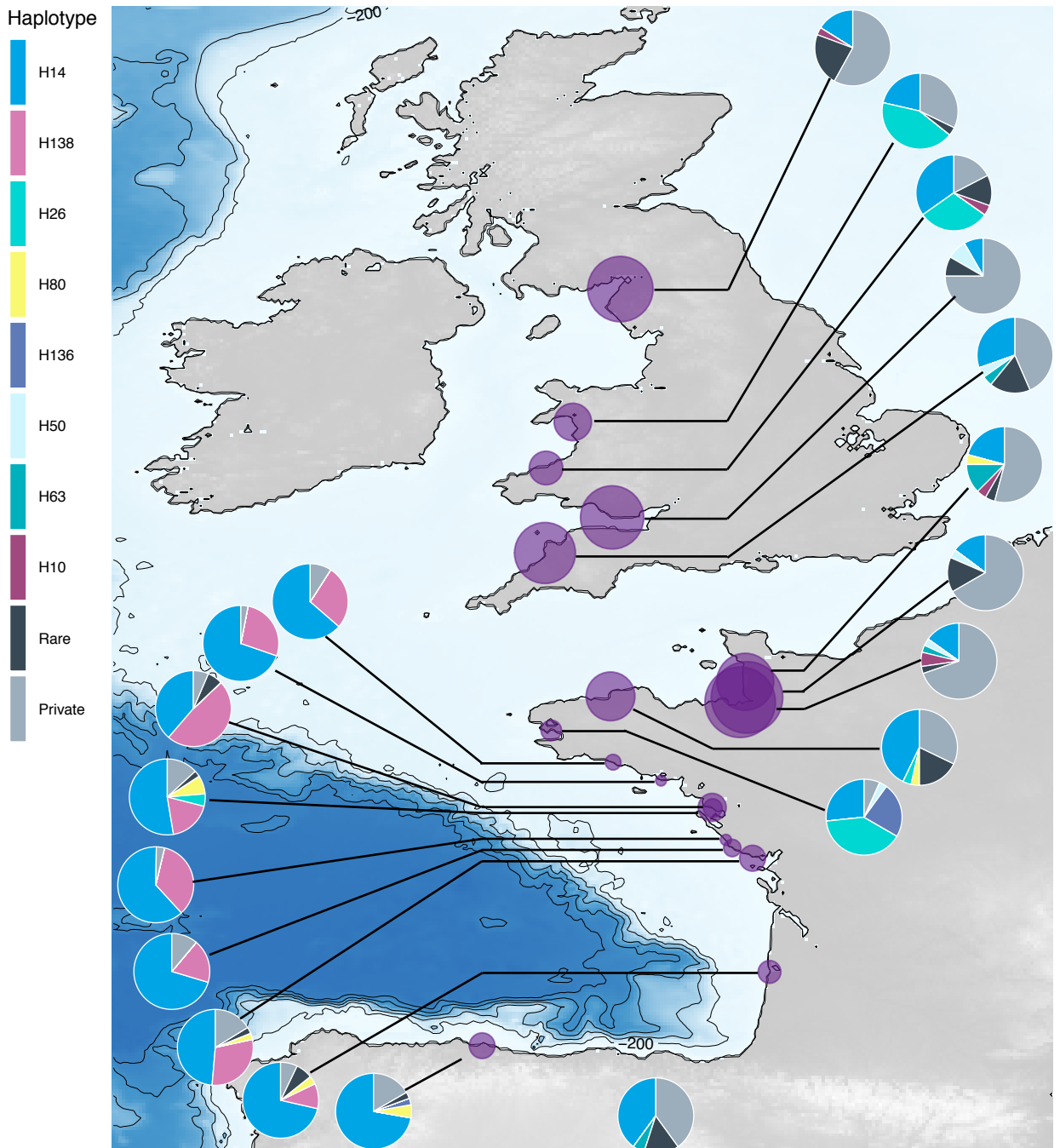


Figure 2



Figure 3

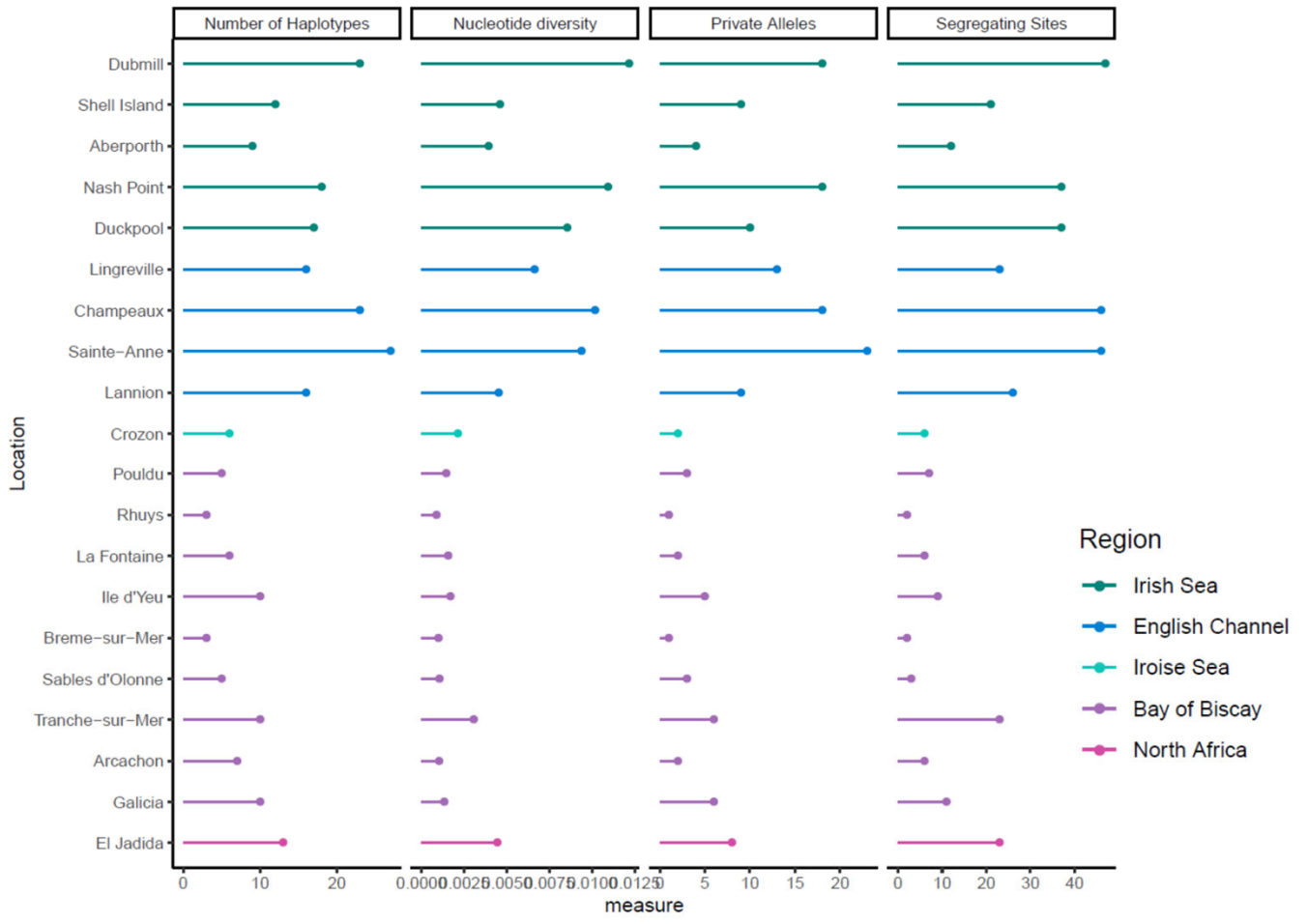


Figure 4

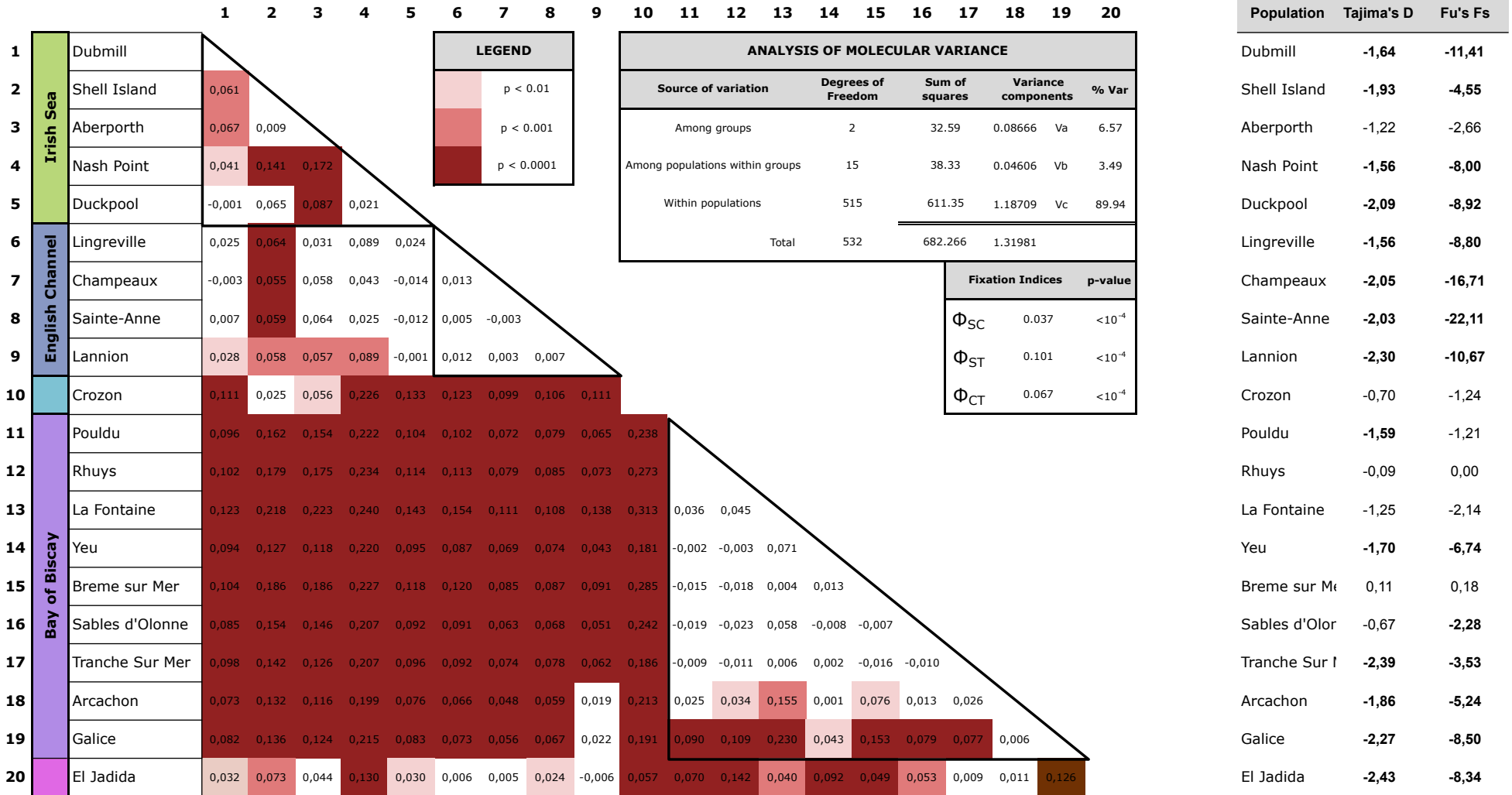


Figure 5

