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ORIGINAL PAPER

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

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Abstract
Species distributions have been profoundly affected by past climate change, and are expected to change considerably in response to future environmental change. To better apprehend how future climate change is likely to affect genetic diversity in marine populations, it is essential to first evaluate the processes that have shaped the current distribution of genetic diversity in the sea. The honeycomb worm is a reef-building polychaete that hosts high biodiversity. Here we show that the genetic diversity in populations of *S. alveolata* is highest towards the edges of the current species range and lowest at its center. Pleistocene glacial cycles likely led to extirpations of *S. alveolata* from central populations in the Bay of Biscay, with coalescent-based estimates of post-glacial colonization dating to the beginning of the Holocene interglacial, from 10,000 to 14,000 years ago. Meanwhile, populations in the Irish Sea and English Channel likely persisted in glacial refugia since the Eemian interglacial, 120,000 years ago. Northern populations host at least two sets of divergent haplotypes, indicating that two refugia possibly existed in the north, with Ireland being a likely second refugium. Within biogeographic regions, populations were overall well-connected, but strong genetic differentiation suggests that little exchange occurs between regions. These two unexpected reservoirs of genetic diversity at the range edges 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 genetic 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 (O'Connor et al. 2012). Rapid environmental change coupled with effective migration may also increase the inflow of maladapted genes selected under very different conditions elsewhere into populations (Frankham et al. 2011). Furthermore, migration among genetically differentiated sources can lead to admixture due to secondary

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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 (Pinsky et al. 2020). The response of species to climate change, therefore, depends on the interaction of multiple factors, and is often difficult to predict.

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 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 as much as 130 m over the Pleistocene not only offset the current coastline offshore, but also altered the configuration and continuity of shallow marine habitats (Lambeck et al. 2002). For example, much of the English Channel was dry during Pleistocene glacials, and the Baltic Sea was covered by the Scandinavian ice sheet (Maggs et al. 2008). 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 summaries, 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 use the common and broadly distributed reef-building polychaete *Sabellaria alveolata* to investigate the response of a foundation species to past climate change throughout much of its distribution in the northeastern Atlantic. 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 spatial as well as long time scales, but presently, *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 (e.g. 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 25°C (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).

Based on the sequencing of the *cox-1* gene from ca. 580 individuals from 20 populations sampled from two biogeographic provinces, the objective of this study was to examine whether: (1) Pleistocene climate change affected the genetic diversity of *S. alveolata*, (2) *S. alveolata* persisted in glacial refugia, and (3) genetic breaks observed in this species correspond to biogeographic partitioning in the northeast Atlantic. We conclude with a discussion on how the answers to these questions can better inform future conservation measures for this important reef-builder.

Methods

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 ten localities in the Lusitanian Province (nine from the Bay of Biscay in France and Spain and one site on the 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.

Molecular analysis

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 the manufacturer's 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 µl and consisted of 2.5 µl of 10X buffer containing $MgCl_2$, 2 µl of 10 mM dNTPs, 0.5 µl of BSA at 1 mg/ml, 1 µM of each of 10 mM primers (LCO-1490 and HCO-2198), 0.1 µl of Thermoprime high fidelity Taq polymerase (ABgene) and 2 µl of template DNA (1:10 to 1:20 dilution of the DNA stock solution). PCR reactions were conducted in an 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 s, annealing at 45°C to 49°C (+ 1°C at each cycle) for 45 s and extension at 72°C for 1 min 10 s, 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 µl and was composed of 0.5 µl of Big Dye, 0.75 µl of 5X Buffer, 0.5 µl of 10 µM forward or reverse primers, 1.25 µl of purified DNA and 1.75 µl 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.

Data analysis

Sequence chromatograms were visually inspected for accuracy of base calls using the ABI Chromas software (Technelysium Pty Ltd.). Bioedit (Hall 1999) 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 sequences over all individuals.

Genetic diversity

Geographic patterns of genetic diversity were examined by calculating haplotype frequencies, the number of haplotypes (N_h), haplotype richness (H_{rar}), 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_{rar}), 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).

Demography and admixture inferences

Assuming neutrality, 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. To examine changes in demography over time in honeycomb worm populations, Extended Bayesian Skyline Plots (Heled and Drummond 2008) were generated in Beast v2.6.3 (Bouckaert et al. 2019) for the Irish Sea + English Channel populations as well as for the Bay of Biscay populations. Two substitution rates, 3.5% and 4.7% per million years, were selected based on recent recalibration of *cox-1* divergence times estimated for polychaete sister species pairs across the Bering Strait (Loeza-Quintana et al. 2019). The Extended Bayesian Skyline Plots were estimated using the HKY substitution model with empirical estimates of the base frequencies, a strict clock model, a population size scale factor of 0.5 (for mitochondrial DNA) and a prior on the mean of the population size as a normal distribution centered on 1 with a standard deviation of 0.1. Three test runs were conducted to select an adequate chain length for the Markov Chain Monte Carlo, at 10^7, 10^8 and 10^9 steps. Full runs used an MCMC of 5×10^8 steps, logged at every 10,000 steps. Log files were inspected for parameter convergence using Tracer v1.7.1 (Rambaut et al. 2018), and the EBSPAnalyzer tool in Beast2 was used to discard the initial 20% of steps and generate a linear reconstruction of the log files for plotting.

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) 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 and genetic distances between populations (Rousset 1997). The matrix of ϕ_{ST} was obtained with Arlequin and 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 (10,000 permutations) in one of the two distance matrices.

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. 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 + North Africa. In all 3 networks, shared haplotypes showed haplotype frequencies over all sampled regions, to facilitate comparisons.

Results

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%) (Figs. 1, 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 (present in a single population). Only seven 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

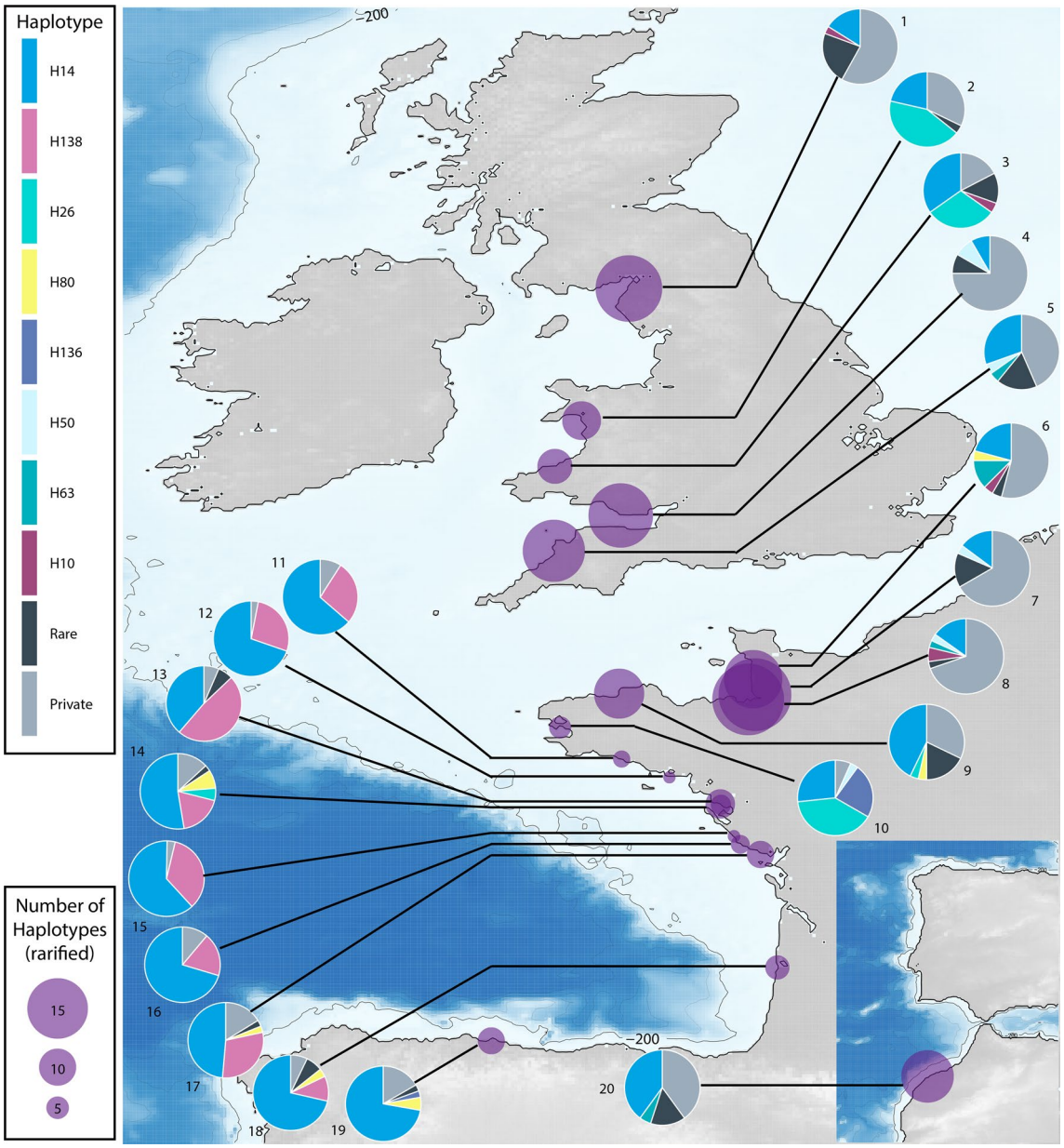


Fig. 1 Map of the 20 sampling locations of *Sabellaria alveolata* collected for this study. The size of the violet circle at each location corresponds to haplotype richness at the site, following rarefaction to account for unequal sample sizes. Haplotype frequencies are shown as pie charts connected to each site by a black line, and numbered 1–20 as in Fig. 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

private haplotypes (fewer than six, whatever the population). The reef located on the Moroccan coast had an intermediate number of eight private haplotypes. The geographical distribution of haplotypes (by population) is shown in Fig. 1.

Haplotype networks were estimated for the Irish Sea, English Channel + Iroise Sea, Bay of Biscay + North Africa (Fig. 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

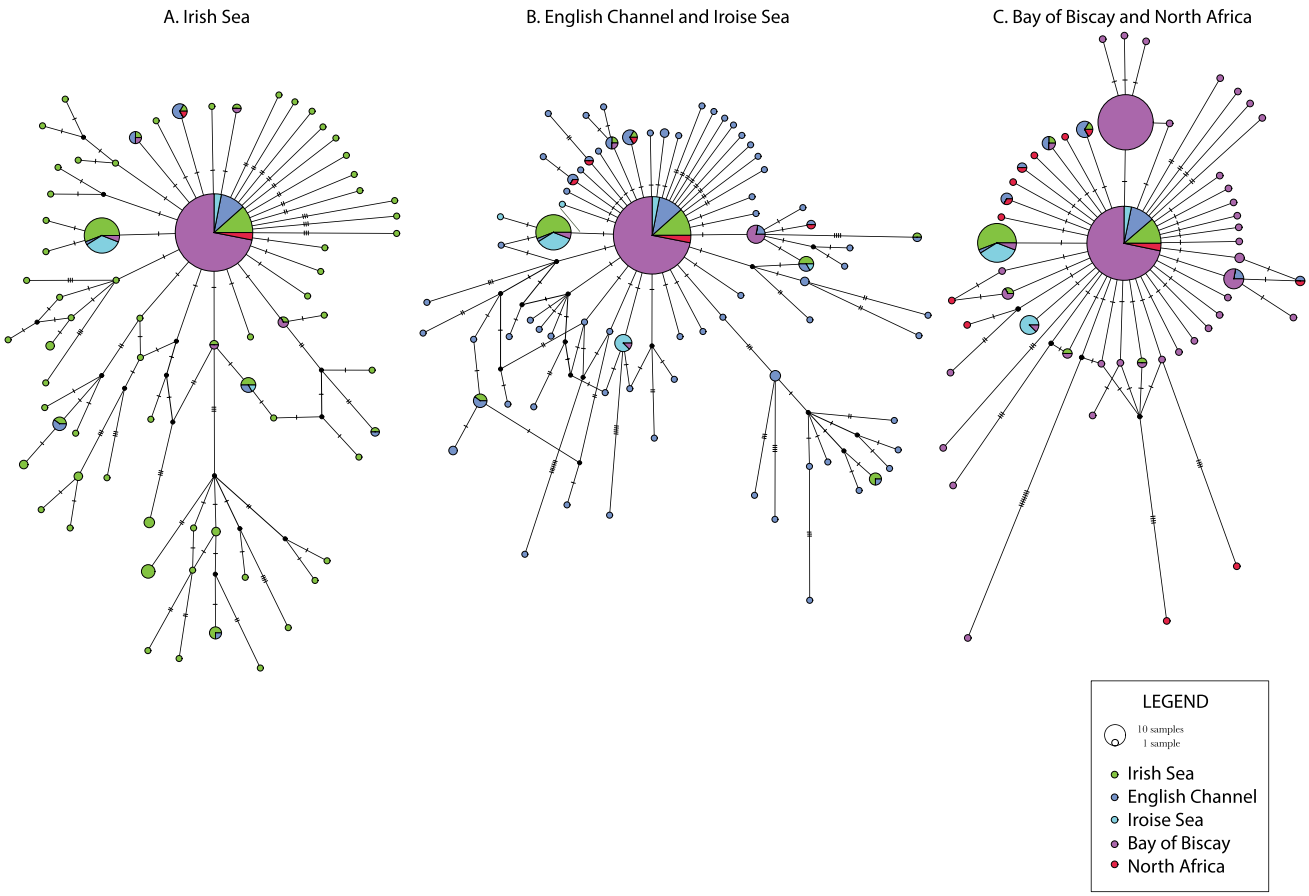


Fig. 2 Haplotype networks for **a** Irish Sea, **b** English Channel and Iroise Sea, **c** Bay of Biscay and North Africa. Haplotypes common to more than one region are shown with the frequencies observed over the whole dataset (all regions). The positions of shared haplotypes were kept constant in all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

in the Bay of Biscay and two on the North African coast (Fig. 2).

Population genetic diversity

At the population level, five indices of genetic diversity all showed greater values in the Irish Sea, English Channel and North Africa than populations of the Iroise Sea and Bay of Biscay (Figs. 1, 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 (Fig. 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 (Fig. 3). The number of private alleles was strikingly different among geographic regions, being as large as 23 in Sainte-Anne (English Channel), but no greater 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 the number of segregating sites ranged from 2 to 23 (Fig. 3).

Population genetic structure

Analysis of molecular variance (AMOVA) indicated significant population genetic structure at all hierarchical levels (Fig. 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,

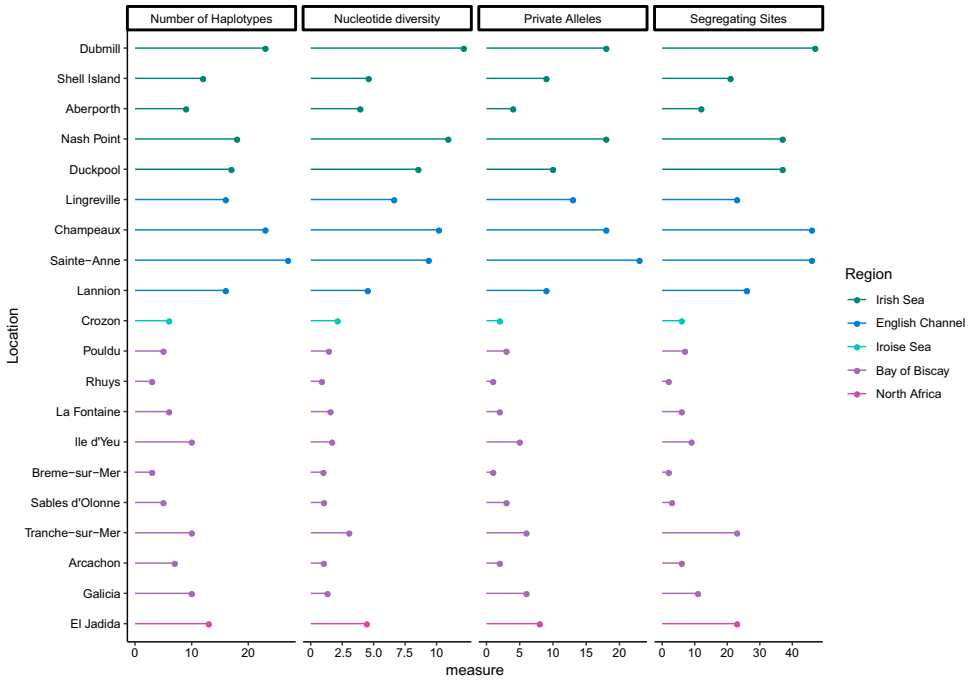
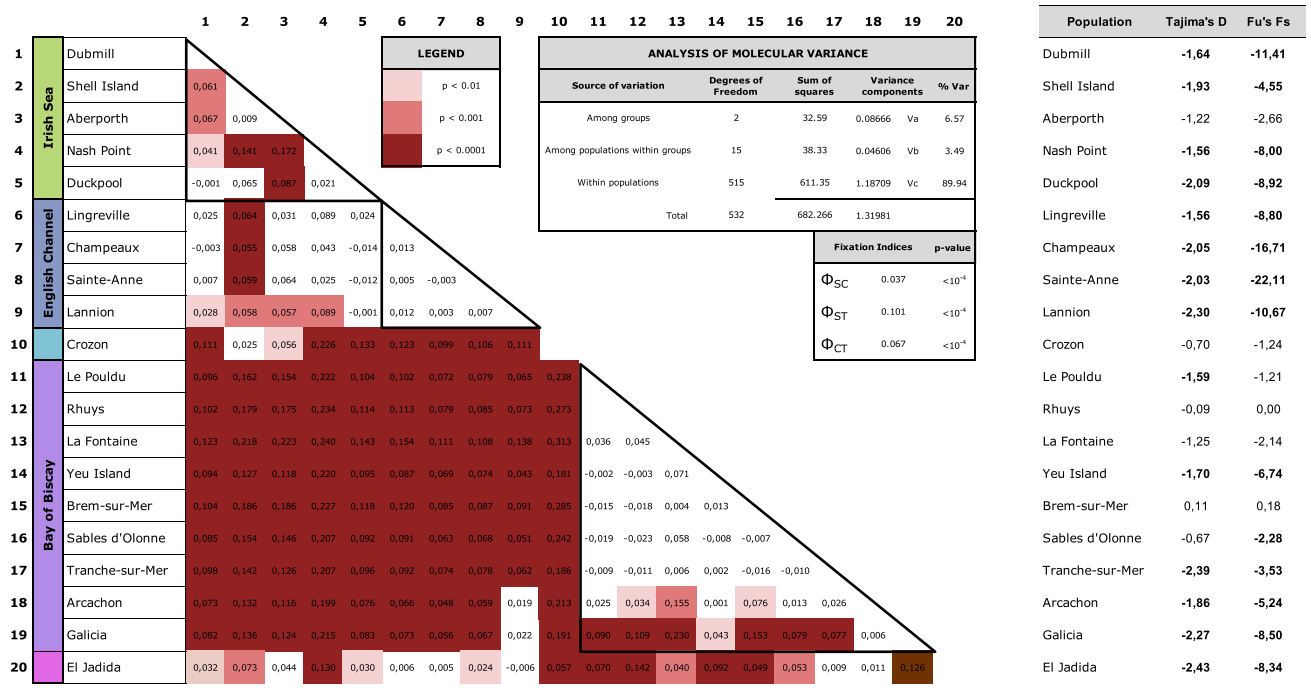


Fig. 3 Molecular diversity indices for each population, including number of haplotypes, nucleotide diversity ($\times 10^3$), number of private alleles and number of segregating sites. Each population is colored according to the region in which it was sampled (see legend)



Population	Tajima's D	Fu's Fs
Dubmill	**-1,64**	**-11,41**
Shell Island	**-1,93**	**-4,55**
Aberporth	-1,22	-2,66
Nash Point	**-1,56**	**-8,00**
Duckpool	**-2,09**	**-8,92**
Lingreville	**-1,56**	**-8,80**
Champeaux	**-2,05**	**-16,71**
Sainte-Anne	**-2,03**	**-22,11**
Lannion	**-2,30**	**-10,67**
Crozon	-0,70	-1,24
Le Pouldu	**-1,59**	-1,21
Rhuys	-0,09	0,00
La Fontaine	-1,25	-2,14
Yeu Island	**-1,70**	**-6,74**
Brem-sur-Mer	0,11	0,18
Sables d'Olonne	-0,67	**-2,28**
Tranche-sur-Mer	**-2,39**	**-3,53**
Arcachon	**-1,86**	**-5,24**
Galicia	**-2,27**	**-8,50**
El Jadida	**-2,43**	**-8,34**

Fig. 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

$\Phi_{SC} = 0.037$, $p < 0.0001$), suggesting genetic drift and/or limited connectivity among populations, even at the regional level.

Pairwise fixation indices (ϕ_{ST}) are given in Fig. 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 strong isolation by istance at the regional level.

Demographic changes

Tajima's 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) (Fig. 4c). 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 (Fig. 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: (1) over all populations, (2) for the Irish Sea, (3) for the English Channel and (4) for the Bay of Biscay (Fig. 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" (Fig. 5a). The profile expected under mutation-drift equilibrium is found in all 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 (Fig. 5d). 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 (Fig. 5b, 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 even more complex distributions, 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.

Extended Bayesian Skyline Plots estimated for the Irish Sea + English Channel as well as the Bay of Biscay populations rejected the null hypothesis of constant population size in both regions, with the number of demographic events excluding zero (i.e., no population expansion), and having a median of one population expansion event in all simulations. Inspection of the log files in Tracer indicated that parameter convergence was reached, with effective sample sizes for estimated parameters ranging from 261 to > 2500. Population expansion was estimated to have taken place in the Irish Sea + English Channel populations between 100,000 and 140,000 years ago, based on mutation rates of 4.7% and 3.5% per million years, respectively (Fig. 6a). For the Bay of Biscay, population expansion was estimated to have taken place between 10,000 and 14,000 years ago, based on the same mutation rates (Fig. 6b).

Discussion

Phylogeography considers the spatial distribution of alleles within a species 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 (Ireland/Scotland), while it is 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 the observed patterns of genetic diversity and demographic history for *S. alveolata*. The Irish Sea and English Channel have higher levels of haplotype and nucleotide diversity than other regions, as well as 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

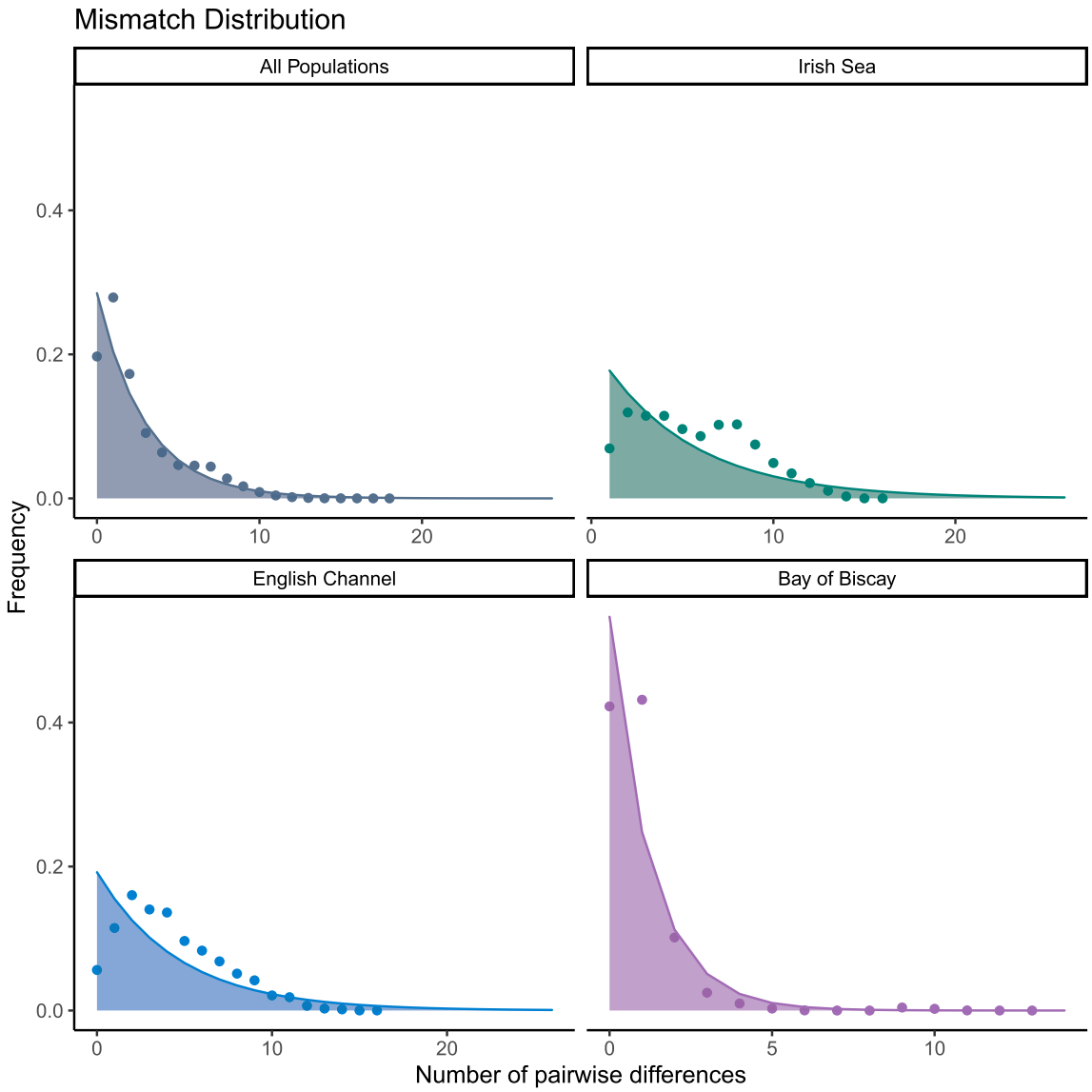


Fig. 5 Mismatch distribution calculated over **a** all populations combined, **b** populations located in the Irish Sea; **c** populations located in the English Channel and **d** populations located in the Bay of Biscay. Observed values of the mismatch distribution are shown as circles, and expected 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

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 previous glacial periods, migrating to other parts of 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 Extended Bayesian Skyline Plot supports this interpretation, as it shows that effective population size has been increasing in this part of the distribution since 100,000–140,000 year ago (Fig. 6a), which corresponds well with the timing of the Eemian interglacial, which started at 120,000–130,000 years ago (Adams et al. 1999). No population bottleneck is detected in the time period that corresponds to the maximum extent of the British-Irish Ice Sheet during the last glacial period [estimated at 27,000 years ago (Clark et al. 2012)], suggesting that honeycomb worm populations persisted in these two regions through the last glaciation. Reconstruction of the ice sheets also suggest that southern Ireland remained ice-free

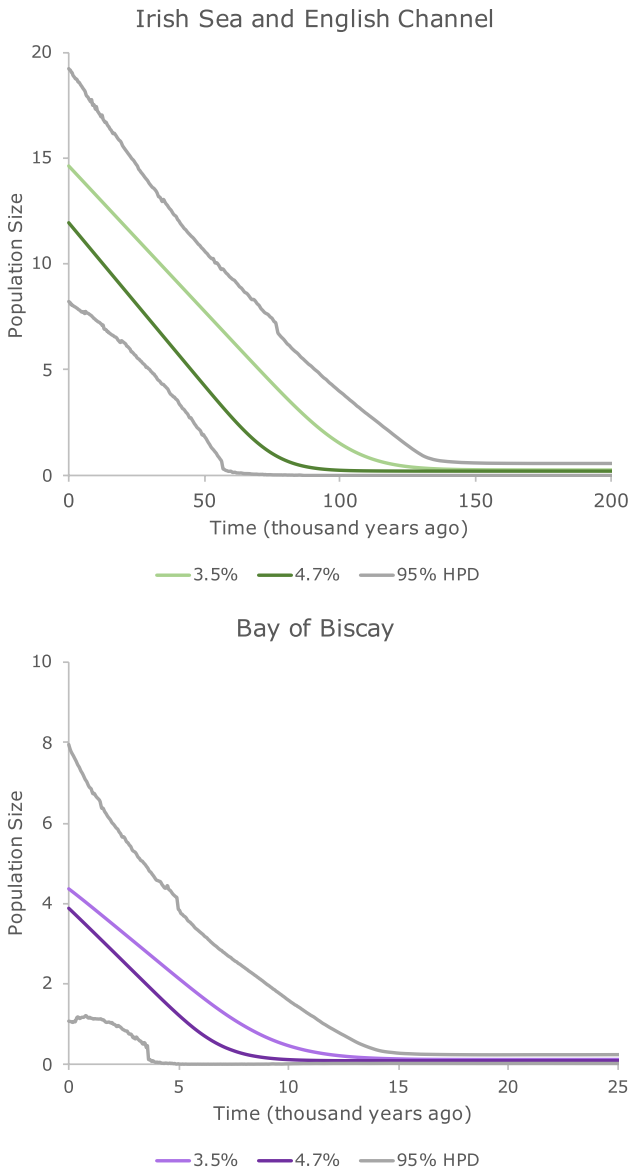


Fig. 6 Extended Bayesian Skyline Plots depicting estimates of effective population size over time for populations in **a** the Irish Sea and English Channel and **b** the Bay of Biscay. Dark colored lines show estimates based on a divergence rate of 4.7% per million years, and light colored lines for a divergence rate of 3.5% per million years. Grey lines represent upper and lower 95% highest posterior density

(Clark et al. 2012) and could have served as a refugium for intertidal species such as *S. alveolata*.

In contrast, honeycomb worm populations in the Bay of Biscay mostly harbor 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. The Extended Bayesian Skyline Plot estimates that population expansion started at 10,000–14,000 years ago (Fig. 6b), which corresponds well with the start of the present interglacial period [the Holocene, 11,000 years ago (Adams et al. 1999)]. Colonization of the Bay of Biscay is, therefore, estimated to have occurred much later than in the Irish Sea and English Channel by honeycomb worms.

While over the entire distribution range, genetic diversity is lowest in central populations, it is interesting to note that within the Bay of Biscay, the highest levels of genetic diversity are observed in Yeu Island, La Fontaine aux Bretons and Tranche-sur-Mer, three central populations. At the regional level, genetic diversity does appear to be greatest in central and lower in marginal populations, as is typically expected. Four haplotypes observed in the Bay of Biscay diverged by more than five mutations from the central haplotype. These haplotypes are likely the result of migration from other regions, but further sampling is required to identify the source populations.

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. Alternatively, human-mediated introduction from northern to southern populations may explain this pattern, as has been proposed for some molluscs (Simon-Bouhet et al. 2006; El Ayari et al. 2017). Genetic diversity in this population in Morocco is also relatively high, and presents a profile of demographic expansion (high H_{rar}, with negative and significant values of Tajima's D and Fu's F_S), which could indicate the existence of another refugium in the southern part of the distribution, which unfortunately remained unsampled in our study, along with populations on the Iberian Peninsula. 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 various macroalgae (Provan and Maggs 2012; Assis et al. 2013, 2018; Nicastro et al. 2013). For example, high genetic diversity in the Iberian coast has been observed for the red alga *Chondrus crispus* (Provan and Maggs 2012) and the brown kelp *Saccorhiza polyschides* (Assis et al. 2013) but both studies show that due to restricted connectivity among Iberian and northern populations, the unique genetic diversity observed in the rear edge populations are threatened by increasing global temperatures and bottlenecks. 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) and 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 some polychaetes and molluscs whose distribution spans the Iroise Sea (Luttikhuizen 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 northeastern 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 northeastern Atlantic marine species.

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

We previously showed that 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 though 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 likely maintained throughout this bay, leading to the homogenization of haplotypes, 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), 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 were 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.

Population genetics and conservation

Our results have a number of implications for the conservation of *S. alveolata*, an important ecosystem engineer. The Irish Sea and English Channel are two important reservoirs of genetic diversity for the species. However, 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* (Firth et al. 2015) resulting in loss of genetic diversity locally. Furthermore, despite increasing sea surface temperatures near the northern range edge, which should favor range expansion, populations in Ireland have remained surprisingly stable over the past several decades, limited by unfavorable hydrodynamic conditions (Firth et al. 2021). 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 are numerous (Curd et al. 2020), 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.

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 programs. Finally, future research that focuses on populations to the south of the Gironde River is required to determine whether these populations are isolated and require specific conservation measures.

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 the extirpation of populations in the Bay of Biscay with subsequent post-glacial colonization taking place only in the Holocene (10,000 - 14,000 years ago), thereby leading to reduced genetic diversity throughout the region. In contrast, the species has persisted for at least 100,000 years in the English Channel and Irish Sea, surviving in at least one glacial refugium over the Last Glacial Maximum. 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 other intertidal marine organisms. 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. In addition, 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. Finally, we identified several areas which should be targeted in conservation programs, to both prevent population collapse and preserve the evolutionary potential of *S. alveolata*, an important ecosystem engineer in the northeast Atlantic. In this context, it is worth noting that 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 honeycomb worm's distribution.

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Author contributions FR, SFD and FV designed the study, FR, FV and SFD collected the specimens, FR acquired the sequence data, FLDN, FV and FR analyzed the data, FLDN led the writing of the manuscript and all authors were involved in the writing process.

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Availability of data and material Newly generated mtDNA sequences have been deposited in GenBank (Accession Numbers: MT955994-MT956576).

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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