EVALUATING SIGNATURES OF GLACIAL REFUGIA FOR NORTH ATLANTIC BENTHIC MARINE TAXA

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Abstract. A goal of phylogeography is to relate patterns of genetic differentiation to potential historical geographic isolating events. Quaternary glaciations, particularly the one culminating in the Last Glacial Maximum ~21 ka (thousands of years ago), greatly affected the distributions and population sizes of temperate marine species as their ranges retreated southward to escape ice sheets. Traditional genetic models of glacial refugia and routes of recolonization include these predictions: low genetic diversity in formerly glaciated areas, with a small number of alleles/haplotypes dominating disproportionately large areas, and high diversity including "private" alleles in glacial refugia. In the Northern Hemisphere, low diversity in the north and high diversity in the south are expected. This simple model does not account for the possibility of populations surviving in relatively small northern periglacial refugia. If these periglacial populations experienced extreme bottlenecks, they could have the low genetic diversity expected in recolonized areas with no refugia, but should have more endemic diversity (private alleles) than recently recolonized areas. This review examines evidence of putative glacial refugia for eight benthic marine taxa in the temperate North Atlantic. All data sets were reanalyzed to allow direct comparisons between geographic patterns of genetic diversity and distribution of particular clades and haplotypes including private alleles. We contend that for marine organisms the genetic signatures of northern periglacial and southern refugia can be distinguished from one another. There is evidence for several periglacial refugia in northern latitudes, giving credence to recent climatic reconstructions with less extensive glaciation.

Key words: climatic change; coalescence; genetic diversity; glaciations; haplotype networks; isolation; mitochondrial markers; recolonization; refugia.

INTRODUCTION

It is clear that the geographical ranges of marine organisms alter as climate changes (Southward 1991, Perry et al. 2005, Lima et al. 2007), but predicting how each species will respond is difficult (Helmuth et al. 2002, Broitman et al. 2008). Distributions during previous climatic events such as the glaciations that dominated the Pleistocene epoch (Willis and Niklas 2004) provide valuable insights into rates of migration and population resilience. Paleoecology has revealed that in temperate Europe and North America terrestrial species experienced repeated cycles of range contraction and expansion during glaciations (Huntley and Birks 1983, Pielou 1991). Nevertheless, the fossil record has some deficiencies such as differential preservation and variable levels of taxonomic resolution (e.g., morphologically indistinguishable cryptic species [Comes and Kadereit 1998]). Genetic data have therefore been pivotal in recent advances in understanding of the evolutionary legacy of the ice ages (Hewitt 1996, 2004).

Because species responded individually to climatic change during glaciations, communities were not stable over time (Hewitt 1996, Comes and Kadereit 1998, Taberlet et al. 1998). During the advances of Northern Hemisphere ice sheets, temperate species became restricted to glacial refugia, "areas where some plants or animals survived an unfavorable period ... when plants

or animals of the same kind were extinguished in surrounding areas" (Andersen and Borns 1994). Combined analyses of paleoecological and genetic data have identified various refugia during the Last Glacial Maximum (LGM), 25–18 ka (thousands of years ago), and reconstructed recolonization routes for some species into formerly glaciated regions (Bernatchez and Wilson 1998, Taberlet et al. 1998). In addition to extensive southern glacial refugia where large (but nevertheless reduced) population sizes were maintained (Taberlet et al. 1998, Hewitt 2004), small periglacial refugia (isolated northern ice-free areas) may have allowed pockets of diversity to persist (Stewart and Lister 2001, Petit et al. 2003, Rowe et al. 2004, Provan and Bennett 2008).

Isolation into refugia reduced geographical ranges and populations, resulting in a characteristic genetic signature of high genetic diversity within and high dissimilarity between refugial populations (Hewitt 1996, 2004, Comes and Kadereit 1998). Leptokurtic dispersal at the "leading edge" of colonization can involve a series of genetic bottlenecks such that recolonized non-glacial areas show low diversity dominated by few genotypes and a high frequency of alleles identical to or descended from the founding population (Hewitt 1996, 1999, 2004, Ibrahim et al. 1996, Bernatchez and Wilson 1998).

Though a good starting point, this model is oversimplified. Northern unglaciated regions, or periglacial refugia, might experience bottlenecks so that their lack of genetic diversity mimics that of recolonized regions (Brochmann et al. 2003). The high diversity characteristic of southern refugial populations could also develop as a result from secondary contact. For example, a Europe-wide analysis of 22 tree species showed that although there was indeed high genetic differentiation between glacial refugial populations, the greatest within-population haplotype diversity was north of the refugia, resulting from the admixture of genetically divergent recolonizers (Petit et al. 2003). When the products of different refugia come into secondary contact there are zones of hybridization between subspecies or lineages that may form concordant multi-species "suture zones" or transition zones (Hewitt 1996, 1999). Thus the distribution of particular haplotypes, including private alleles (those endemic to populations or regions), is important in distinguishing between refugial populations and recently recolonized areas. The relationship between allelic richness and a measure of divergence between haplotypes can also be a useful indicator of refugial status (Petit et al. 2002).

Although many marine species show large-scale dispersal (Lessios et al. 1998), as well as remarkable large-scale structure (Palumbi 1994, Sotka et al. 2004), much remains to be learned about the ability of marine organisms to track suitable habitat during climatic changes (Roy et al. 2001, Wares and Cunningham 2001). In the future, we are certain to see continued distributional changes with effects on levels of biodiver-sity as species retreat from formerly suitable habitat (Walther et al. 2005).

When comparing glacial refugia in marine and terrestrial systems, it is important to remember that marine environments during Pleistocene glaciations experienced rapid changes in sea level. During the LGM, the transfer of water to land-based ice sheets caused sea level to drop rapidly, by 30–40 m within 1000–2000 years, to a lowstand of approximately −130 m (Lambeck et al. 2002). During lowstands, coastlines of the North Atlantic Ocean and its indentations retreated seaward by up to several hundred kilometers (Fig. 1). Post-glacial sea level rise has obscured the fossil record (Marko 2004), making it difficult to directly establish species' distributions at the LGM.

Here, we provide a comparative genealogical meta-analysis based on data from published studies of North Atlantic benthic marine taxa, reanalyzed in a common framework. Our main aim is to compare observed patterns of phylogeographic structure with the expectations of current models of genetic signatures of glaciations, with particular reference to the detection of potential refugia. Because interpretations of the precise extents of ice sheets and their timing are controversial (Fig. 1; Sejrup et al. 2005), and knowledge of past biotas can contribute to reconstructions of glaciation, we highlight examples where our analysis clarifies glacial histories. Clearly, there are limitations in that the repeated Quaternary glacial–interglacial cycles complicate the search for genetic signatures of isolation in refugia (Jesus et al. 2006): the effects of recent events, including the post-LGM Younger Dryas cold spell, have been superimposed onto patterns of older isolation.

SETTING THE SCENE: NORTH ATLANTIC GLACIATIONS AND POTENTIAL MARINE REFUGIA

The Last Glacial Maximum

The last major glacial cycle started about 116 ka, and the last advance, the LGM, occurred around 21 ka (Mix et al. 2001, Pflaumann et al. 2003 [all dates are calibrated/calendar years BP]). The extent of the major ice sheets at the LGM in parts of the North Atlantic region is uncertain (Fig. 1, solid fill [Lambeck et al. 2002, Pflaumann et al. 2003, Sejrup et al. 2005]). Sea surface temperature (SST) reconstructions also vary: CLIMAP (1981) indicated permanent sea-ice cover in the high-latitude North Atlantic, whereas Pflaumann et al. (2003) and Meland et al. (2005) suggest open water at least seasonally. Glacial histories, including past temperature regimes and positions of old shorelines, differ greatly between the eastern and western coastlines of the North Atlantic. Here we describe the environment of the North Atlantic margins during and after the LGM, with particular focus on the possible locations of coastal glacial refugia.

Eastern North Atlantic/Mediterranean potential refugia

At the LGM, the Scandinavian Ice Sheet reached the continental margin and may have fused with the British
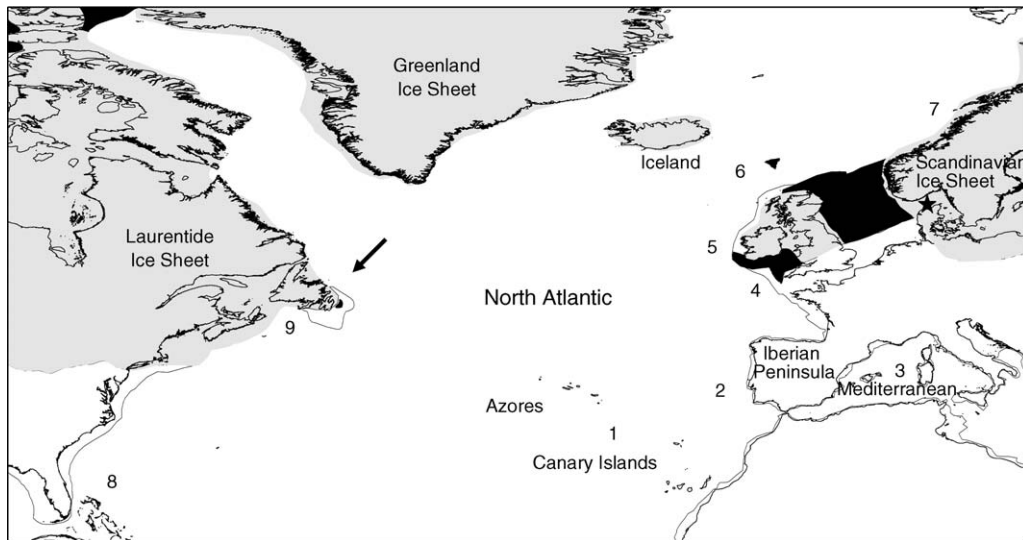
Fig. 1. Northern North Atlantic Ocean at the Last Glacial Maximum. Ice sheets are shown in gray, and solid fill indicates the areas of contention (the tip of Newfoundland, arrowed; the Faroes; southwest of the British Isles; and most of the North Sea, with a deep trench indicated by a star) in contrasting maximum and minimum CLIMAP models (Bowen et al. 2002, Clark and Mix 2002, Dyke et al. 2002, Pflaumann et al. 2003). The paleocoastline (a finer line) and ice sheets were drawn in ArcView 9.4 (ESRI, Redlands, California, USA) from shapefiles at ⟨http://lgb.unige.ch/~ray/lgmveg/index.html⟩, modified following Pflaumann et al. (2003). Potential marine glacial refugia are numbered as in *Setting the scene: North Atlantic glaciations and potential marine refugia.*

Ice Sheet, which covered most of Scotland, England and Ireland (Fig. 1). During the Younger Dryas cold phase (12.8–11.5 ka) the southern limit of sea ice along European coasts is controversial, possibly extending as far south as the Bay of Biscay (Zaragosi et al. 2001). By ca. 12 ka the relative world sea level was −65 m, and a rapid rise continued until ca. 7 ka (Behre 2007). The English Channel was gradually flooded, and a connection to the North Sea was established ca. 9 ka (Behre 2007). The Baltic basin was permanently connected to the sea and became a brackish-water basin by ca. 8.5 ka (Bergland et al. 2005).

Seven potential LGM marine glacial refugia have been identified in the eastern North Atlantic and Mediterranean (Fig. 1, numbered), based on various lines of marine and coastal terrestrial evidence:

1) Azores, Canary Islands, and NW Africa. Many temperate seaweed species are believed to have been restricted to the Canary Islands and southward during the LGM (van den Hoek et al. 1990). The Azores were little affected by the LGM (Morton and Britton 2000, Rogerson et al. 2004). Genetic data provide strong evidence that the Azores were a refuge for the thornback ray *Raja clavata* (Chevolot et al. 2006).

2) Iberian Peninsula. This area south of the ice sheets (Fig. 1) was one of the four terrestrial southern refugia for trees (Petit et al. 2003). Genetic data shown below suggest that the Atlantic coast was a marine refugium for brown algae (Hoarau et al. 2007) and the green crab *Carcinus maenas* (Roman and Palumbi 2004).

3) Mediterranean Sea. Sea level dropped by 110–150 m (Lambeck and Purcell 2005) but the connection to the Atlantic was not broken (Flores et al. 1997); during the

transition to interglacial conditions there was westward upwelling flow (Bigg 1994, Patarnello et al. 2007).

4) Western English Channel. Sedimentary coastlines with river deltas had retreated far to the west (Brault et al. 2004), but deep trenches (e.g., Hurd Deep) may have remained marine. Genetic data discussed below for red and brown algae are consistent with a refugium in or near this area (Provan et al. 2005, Hoarau et al. 2007).

5) Southwest Ireland. Although ice may have extended south to southwest England (Fig. 1; Hiemstra et al. 2006), geomorphology and fossil evidence indicate that southwest Ireland was partly unglaciated (Lambeck and Chappell 2001, Stewart and Lister 2001, Bowen et al. 2002). Genetic data support a marine refugium in southwest Ireland for red and brown algae (Provan et al. 2005, Hoarau et al. 2007).

6) Iceland and the Faroe Islands. Although both island groups are thought to have been covered by separate ice caps (Sejrup 2005), a divergent Icelandic population of the isopod *Idotea balthica* (Wares 2001) is consistent with a refugium in Iceland or the nearby Faroes. Genetic data discussed below support a Faroes refugium for the green crab (Roman and Palumbi 2005).

7) Northern Norway. The Lofoten coast was deglaciated about 22 ka (Vorren et al. 1988), and terrestrial fossils (Larsen et al. 1987, Alm and Birks 1991) lend credence to a terrestrial refuge, but we have found no good evidence for marine species.

Western North Atlantic potential refugia

At the LGM the Laurentide Ice Sheet extended eastward out onto the continental shelf with its southern
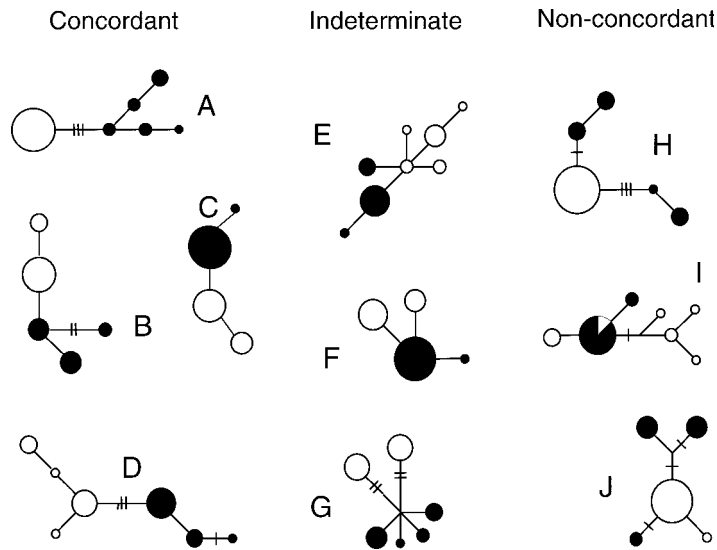
Concordant   Indeterminate   Non-concordant

FIG. 2. Haplotype networks for a simulated case history of ancestral panmixia followed by geographic isolation between two regions (black, white). Coalescent simulations (10 runs) were performed in ms (Hudson 1990) for 10 alleles sampled from each population. The exponential distribution of times between nodes on the simulated tree, with parameter θ, defines the diversity in the population as $xN_e\mu$, with N_e being effective population size, μ the mutation rate, and x the scalar that depends on whether it is a haploid gene ($x = 2$) or a diploid gene ($x = 4$). Mutations are then randomly distributed on the tree according to a Poisson distribution. The longer the branches separating two individuals, the more likely are mutations on those branches. This simulation was carried out with isolation originating $0.8N_e$ generations back in time with θ as $4N_e\mu = 1.0$, and equal population sizes. The settings were chosen to show that a known process leads to variable results, and the values are those that could be encountered in reality: for example if an infinite-alleles model is applied and μ is 1×10^{-5} then N_e is about 25 000, and the time of separation is about 20 ka ($0.8N_e$). The 10 output results have been ordered from fully concordant (networks A–D) through indeterminate (E–G) to non-concordant (H–J).

margin at Long Island (Fig. 1). The RSL drop of 100–135 m (Stea et al. 2001, Clark and Mix 2002) exposed large portions of the continental shelf (Fig. 1) though the precise extent of the ice and the pattern and timing of deglaciation along this complex coastline are controversial (e.g., Josenhans and Lehman 1999, Stea 2001). Land was mostly covered by ice (Stea et al. 2001, Dyke et al. 2002), with coastal refugial areas (Fig. 1, numbered):

8) South of the ice sheets along the Carolinas and southward into Florida and the Gulf of Mexico. This is assumed to have been the main (southern) refugial area for marine taxa, although the general lack of hard substrata may have eliminated many species (Lüning 1990).

9) Atlantic Canada. The GLAMAP 2000 reconstruction has most of the Grand Banks unglaciated (Pflaumann et al. 2003; Fig. 1, arrow), in contrast to Shaw (2003). One or more refugia in Atlantic Canada has been suggested for the anadromous rainbow smelt (Bernatchez 1997). Genetic evidence for a Nova Scotia refugium for a hermit crab (Young et al. 2002) is discussed in *Haplotype diversity and haplotype distribution for North Atlantic species: Model III. Two or more refugia-derived populations with little or no contact.*

GENETIC CONSEQUENCES OF VICARIANT ISOLATION

The role of genetic drift in generating divergent populations

Slow mutation rates of organellar markers mean that most polymorphisms involving more than one base difference certainly pre-date the LGM (Anderson et al. 2006). Most lineage splits are also much older than the LGM. Differential distributions of haplotypes between isolated geographical areas are therefore expected to represent either longer-term isolation or the effects of sorting by genetic drift, with some contribution from haplotypes derived by new mutation.

When a situation of panmixia is ended by glacial isolation into northern and southern refugial populations, each haplotype will drift randomly to higher or lower frequency in each population and rare haplotypes will likely drop out in one or both refugia. The speed and outcome of the process will be affected by population size, which is likely to be higher in a southern refugium. The two refugial populations will not at first be reciprocally monophyletic because of retention of ancestral haplotypes. Finally, enough pre-existing haplotypes go extinct in one of the refugia to create reciprocal monophyly (Neigel and Avise 1986). To demonstrate the wide range of possible outcomes of isolation from ancestral panmixia, because of the strong stochastic element in lineage survival or extinction, we present a simulation (Fig. 2). The results of geographic isolation between two regions (black, white) are explored using a coalescent approach (methods described in legend). The results are presented as parsimony networks in which each circle represents a haplotype (allele), with circle size being proportional to the numbers of individuals with that haplotype; relationships between haplotypes are shown by the lines joining them, with slashes indicating mutational steps separating them (Avise 2000). Several of the networks (Fig. 2A–D) show geographical concordance (black haplotypes forming distinct reciprocally monophyletic clusters relative to white clusters). These simulations have proceeded far enough to have very
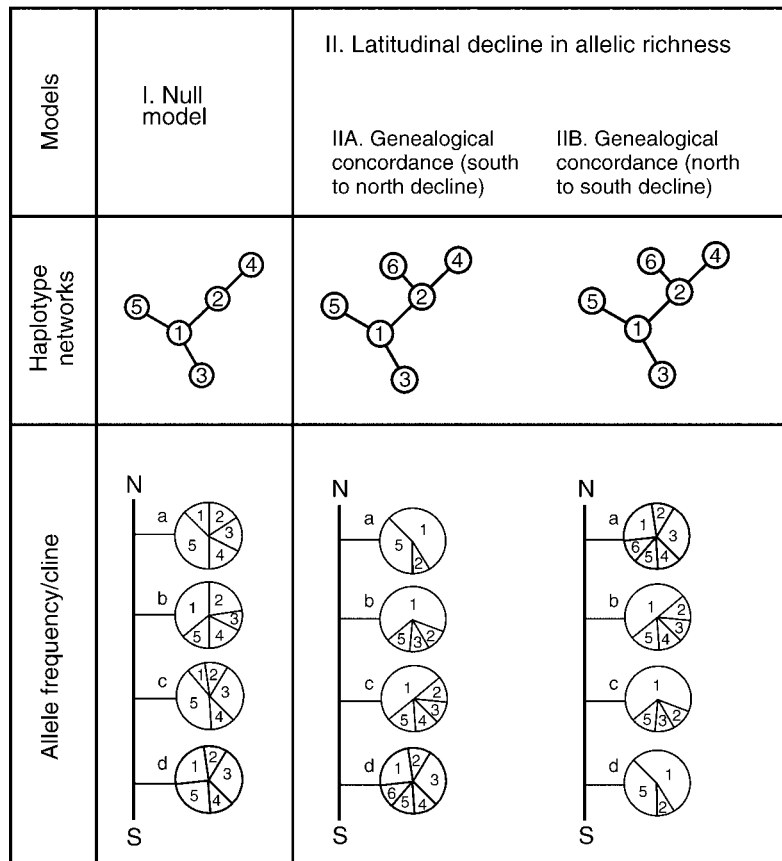
FIG. 3. Models of haplotype networks and haplotype (or allele) frequency distributions that are expected to result from various scenarios involving one or more glacial refugia and the admixture or secondary contact of colonists emerging from these refugia. For each model, we show a haplotype network with 5–6 haplotypes, and the frequency of each haplotype for four populations (a–d) along the coast from north (N) to south (S). In each case, haplotype 6 is a private allele restricted to a refugial population. In Model I, there is no change in haplotype frequency with latitude. In Model II, there is a latitudinal cline in allelic richness, either decreasing northward from a single southern refugium (Model IIA), or southward from a northern refuge for species confined to the north during warm periods (Model IIB). In Model III, two or more refugia have no contact; the northern refugium (haplotypes 1, 2) has lower diversity than the southern refugium (haplotypes 3–6) because its population size was reduced relative to the southern one. We have divided this model into two scenarios, depending on whether the distinct refugia-derived populations show genealogical concordance with the geographical separation (IIIA) or not (IIIB), as explained in Fig. 2. In Model IV, the highest diversity is mid-latitude due to secondary contact between the recolonists from the northern and southern refugia, and the network is (IVA), or is not (IVB), concordant with the distribution of the haplotypes.

few shared haplotypes remaining (there is one example in Fig. 2I). Some networks (E–G) are not fully concordant and others (H–J) are non-concordant (polyphyletic) due to incomplete lineage sorting (Avise 2000). Although in general the two populations are allelically and statistically distinguishable, polyphyletic genealogies mean that we must be cautious in interpreting gene trees that appear to support multiple ancestral refugia.

These expectations assume selective neutrality in the markers used to reconstruct the historical distribution of lineages, an assumption that may often be violated even for cytoplasmic markers (Ballard and Kreitman 1995, Rand 2001, Bazin et al. 2006). Though phylogeographic approaches can identify the initial causes of allopatry among populations (e.g., distinct glacial refugia), we

must also consider factors that maintain regional distinctions during interglacial periods, such as physical, environmental or selective mechanisms that may limit gene flow among populations.

Genetic signatures of refugial scenarios

Competing interpretations of possible origins of high genetic diversity within populations and high divergence between populations need to be evaluated carefully if we are to have any success in identifying glacial refugia and understanding post-glacial colonization processes. We therefore provide models of haplotype networks and allele/haplotype frequency distributions (Fig. 3) that are expected to result from various scenarios involving one or more glacial refugia and the admixture or secondary contact of colonists emerging from these refugia. These
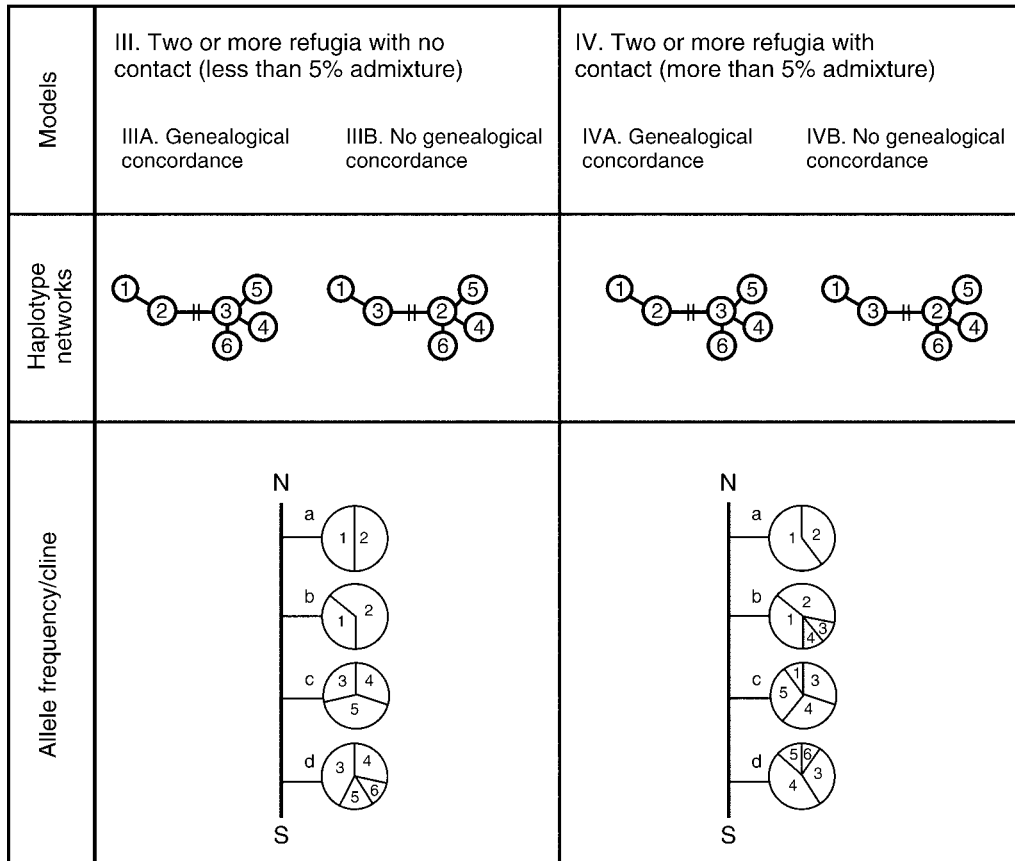
Fig. 3. Continued.

concepts were originally explored in Fig. 4 of Avise et al. (1987). In general, central haplotypes in networks are interpreted as ancestral and occur in high frequency (Avise 2000). Further evidence of a refugium is the presence of haplotypes found in no other population (Slatkin's "private alleles," e.g., haplotype 6 in Fig. 3). A recolonized region can have either low diversity (Models II, III), or high diversity (Model IV) depending on whether it is derived from a single population expanding its range, or from the admixture of two populations, but is expected to have a low proportion of private haplotypes.

Haplotype Diversity and Haplotype Distribution for North Atlantic Species

Choice of taxa and methodology used

The phylogeography of eight well-studied North Atlantic species (or cryptic species) of marine benthic vertebrates, invertebrates and algae is critically compared here with the predictions of the scenarios of glacial refugia described above (Fig. 3). The eight organellar data sets were selected on the basis of adequate geographical sampling and availability of data for comparable molecular markers. For each species (Figs. 4–10), we show relative haplotype diversity across the range, along with haplotype networks, haplotype distributions, and proportions of private alleles (see legend to Fig. 4 for details of methodology). We interpret these with respect to potential glacial refugia, post-glacial recolonization, and competing explanations of variation in diversity, attributing each species to one of the models in Fig. 3. Each species has an individual geographic distribution of haplotype diversity, but there is some concordance between the patterns. Although we do not present any demographic analyses here, published results of such analyses are cited. Other North Atlantic marine organisms with patterns of genetic diversity corresponding to these models are also discussed.

Model I. Null model (panmixia)

None of our selected species exhibits this pattern of genetic diversity. The prime example of panmixia is the migratory European eel (*Anguilla anguilla*). Despite some indications that its genetic structure may be characterized by isolation-by-distance (e.g., Wirth and Bernatchez 2001), recent findings of greater temporal than spatial genetic variation provide support for panmixia in *A. anguilla* (Dannewitz et al. 2005).
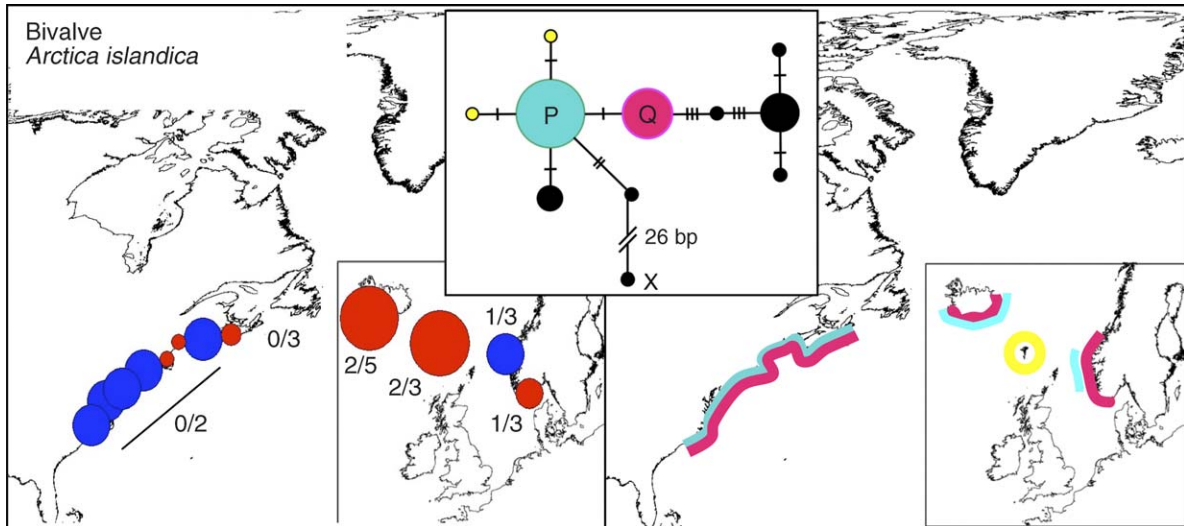
Fig. 4. Diversity plot and haplotype distributions for subtidal bivalve *Arctica islandica* cytochrome *b* sequences (Dahlgren et al. 2001). For this and for Figs. 5–10, maps on left show intrapopulation haplotype diversity after rarefaction resampling, calculated using RAREFAC (written by R. Petit) to account for differences in population sample sizes. Only populations with at least nine individuals sampled were analyzed. In the bubble plots, circle sizes are proportional to deviation from the mean for all populations of that species; red fill indicates diversity above the mean, and blue fill shows diversity below the mean so that the least diverse sample is indicated by the largest blue circle and the most diverse sample by the largest red circle. The proportion of private haplotypes for that population (number of private haplotypes/total number of haplotypes) is shown beside each bubble or bracketed set of bubbles. Maps on right show distributions of key haplotypes or clades, color-coded to the haplotype network (black fill is the default, and the distribution of black-coded haplotypes is not shown). Networks were either made manually (for simple networks such as *A. islandica* [Dahlgren et al. 2001]) or generated using the programs TCS (Clement et al. 2000) and Network (Bandelt et al. 1999).

Model II. Latitudinal cline in allelic richness

Interestingly, none of our species shows a pattern of genetic diversity resembling Model IIA, Hewitt's "northern purity, southern richness" recolonization from southern refugia (but see discussion in Model III of a hermit crab with genetic structure that can be mistaken for Model II). Although examples seem to be rare in the northeast Atlantic, perhaps because of the complex coastline, a cryptic species of the red algal genus *Bostrychia* ("lineage 5") exhibits this genetic structure on American coasts (Zuccarello and West 2003, Zuccarello et al. 2006). High diversity in Florida and Georgia declines through South Carolina and again northward to Connecticut, with private haplotypes at two Florida sites. There are several examples matching Model IIA in the northeast Pacific, including the dogwhelk *Nucella ostrina* (Marko 2004) and the soft coral *Balanophyllia elegans* (Hellberg 1994). In these taxa, northern haplotype diversity is low, without inter-population differentiation or private alleles (Hellberg 1994, Marko 2004).

The subtidal bivalve *Arctica islandica* (Fig. 4) shows an inverse latitudinal cline in genetic diversity (Fig. 3, Model IIB). The highest genetic diversity is in Iceland and the Faroes (Fig. 4), decreasing southward on both coasts of the North Atlantic, where this species becomes confined to deeper water (Dahlgren et al. 2001). Despite small sample sizes, two private haplotypes are found in

the Faroes and two in Iceland, consistent with a refugium in the area of Iceland and the Faroes, with southward expansion toward Norway and Sweden. The low diversity in the western Atlantic, with only the two common haplotypes P and Q at most sites (Fig. 4; haplotype X from Nova Scotia is interpreted here as a cryptic species), corresponds with the predictions for recolonization of this area from a northern European refugium (Fig. 4).

Model III. Two or more refugia-derived populations with little or no contact

In the green crab *Carcinus maenas*, high genetic diversity is observed in populations in three areas (North Sea, English Channel, Southern Iberia) and low diversity in Iceland/Faroes (Fig. 5; Roman and Palumbi 2004). With a general reduction in diversity in northern populations, this could be interpreted as a result of colonization from the South (Model IIA). However, *C. maenas* is considered as an example of IIIA because the haplotype network (Fig. 5) shows three distinct geographically localized clades: (1) a boreal clade distributed mostly in the eastern North Sea, (2) a Faroes/Iceland clade, and (3) a small southern clade distributed widely in southern Europe. Roman and Palumbi (2004) interpreted these deeply diverged clades as evidence of separate refugia. The boreal clade could indicate a refugium in the general area of the North Sea, or else this clade has become extinct in the southern
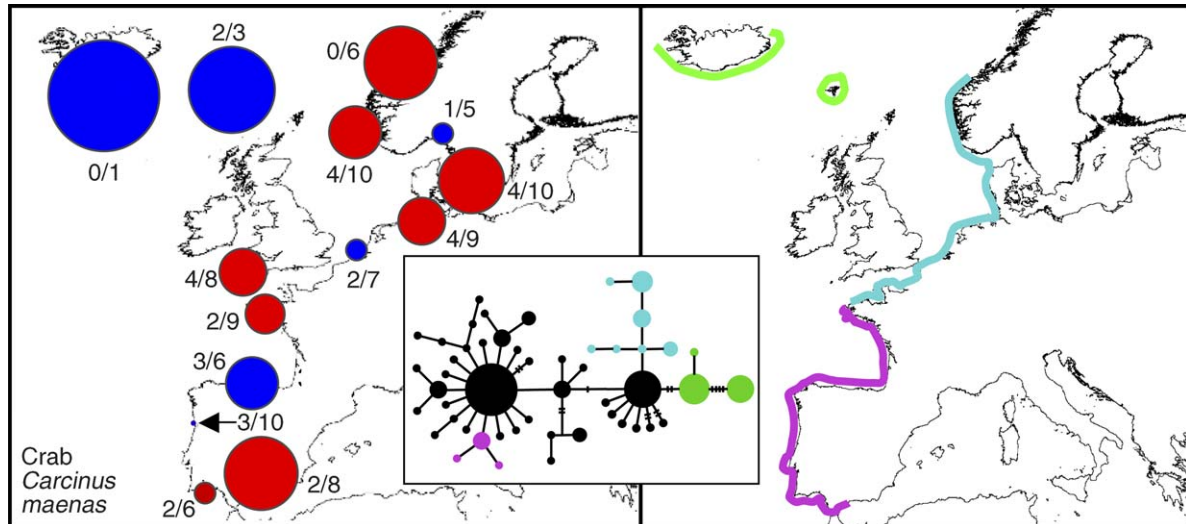
Fig. 5. *Carcinus maenas* (green crab), based on analyses of cytochrome *c* oxidase I (COI) sequences in Roman and Palumbi (2004). See Fig. 4 legend for explanation of bubble plots and colors.

range. If the ice sheets did not cover the region south of Norway a deep trench (see Fig. 1, star) could have served as a small periglacial marine refugium. Long-term separation of populations in Iceland/Faroes from European Atlantic coasts (Roman and Palumbi 2004) is consistent with a northern refugium in the Faroes, which were beyond the ice sheets (Fig. 1) although probably with a small ice cap. The two fixed substitutions between the Iceland/Faroes clade and the rest of Europe are consistent with about 200 ka of divergence. The Icelandic population may have been recolonized from the Faroes, since the Faroes have two private alleles, while Iceland has none at all. A northward expansion into Iceland from the Faroes may also have occurred in the isopod *Idotea balthica*, which has a distinct, monophyletic Icelandic clade (Wares 2001). To summarize, the low haplotype diversity for *Carcinus* in the Faroes shows that care must be taken in assuming that low diversity indicates a recolonized area (Fig. 5).

Our two selected algal species, the red seaweed *Palmaria palmata* and the brown seaweed *Fucus serratus,* show a high degree of concordance in their phylogeographic structure (Fig. 6; Provan et al. 2005, Hoarau et al. 2007). For both species the highest genetic diversity is in the western English Channel, with high but lower diversity in western Ireland. Both species have private haplotypes in Ireland and in the western English Channel. There is partial genealogical concordance with the geographic haplotype distributions (Model IIIA/B). These patterns cannot be interpreted as resulting from secondary contact (Model IVA) because of the presence and proportions of private haplotypes (Fig. 6, colored arrows for *Palmaria* and proportions in each population for *Fucus*) against a background of dense sampling throughout the entire geographical ranges. Although Channel coastlines had retreated far to the west at the

LGM (Fig. 1), enigmatic deep incisions in the seabed such as the Hurd Deep could have been marine lakes, probably seasonally covered with ice and with reduced surface salinity.

The concordant evidence for a western Ireland marine refugium may shed light on a glacial controversy. It implies that the ice sheets did not cover the entire west coast of Ireland during the LGM (Fig. 1), so indications of offshore glaciers (Sejrup et al. 2005) date from earlier glaciations. Rapid northward and southward recolonization sweeps from these regions are inferred from distributions of two common haplotypes in both *Palmaria* and *Fucus* (Fig. 6; Provan et al. 2005, Hoarau et al. 2007). These exemplify the low diversity expected at the leading edge of colonization (Ibrahim et al. 1996). It seems possible that the opening up of vast areas of new habitat following postglacial sea level rise resulted in rapid marine recolonization. For *Fucus*, a third, Iberian refugium at the present southern distribution limit is indicated by a southern geographic clade (shown in green). Coalescent analyses for the two species produce similar estimates of population expansion during the last interglacial period 128–67 ka (Provan et al. 2005, Hoarau et al. 2007). In *Palmaria*, a distinct mitochondrial lineage in North America and Iceland points to a northern/western periglacial refugium that cannot be localized at present but could be the ice-free coastal shelf of southeast Greenland (Brochmann et al. 2003).

For the hermit crab *Pagurus longicarpus*, diversity is high in the Gulf of Mexico and very low in New England and Nova Scotia (Fig. 7; Young et al. 2002). The Gulf of Mexico is clearly the main southern refugium but despite the low diversity to the north, there is evidence from haplotype distributions for periglacial refugia. The divergence between Maine and Nova Scotia was
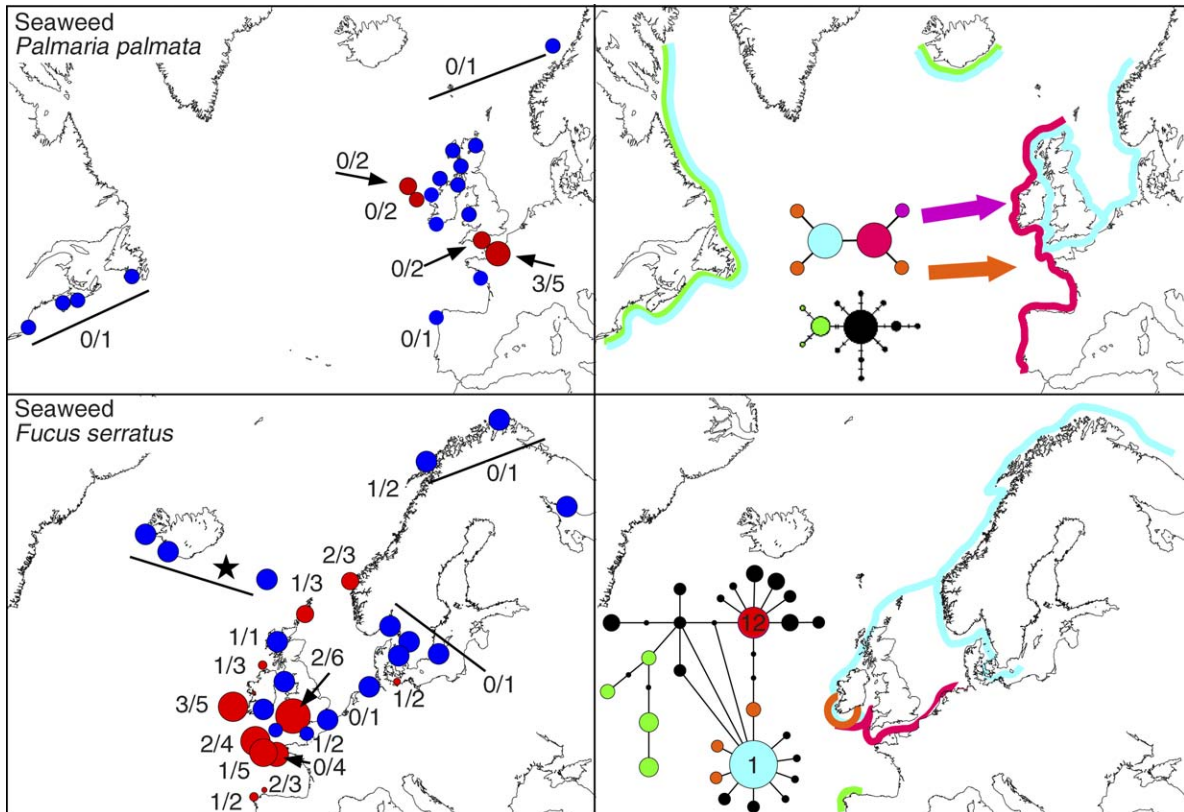
FIG. 6.   Two seaweed species. (Top panels) Red seaweed *Palmaria palmata,* based on Provan et al. (2005), showing plastid PCR-RFLP diversity (upper network) with distribution of plastid haplotypes (pink and turquoise lines) and arrows indicating location of particular haplotypes. Distribution of North American and Icelandic mitochondrial cox2-3 spacer clade (lower network) is shown as a green line. (Bottom panels) Brown seaweed *Fucus serratus*, based on mitochondrial marker in Hoarau et al. (2007). The star indicates that Faroes and Iceland populations are introduced (Coyer et al. 2006). See Fig. 4 legend for explanation of bubble plots and colors.

estimated by Young et al. (2002) to pre-date the LGM, suggesting that the Nova Scotian population might represent the descendants of a northern glacial refugium. This distribution is unlikely to represent recolonization

from the south, because neither of the two haplotypes in Nova Scotia is shared with southern populations. Insufficient time has elapsed for genetic drift to have pruned the haplotype network to a pattern of geograph-
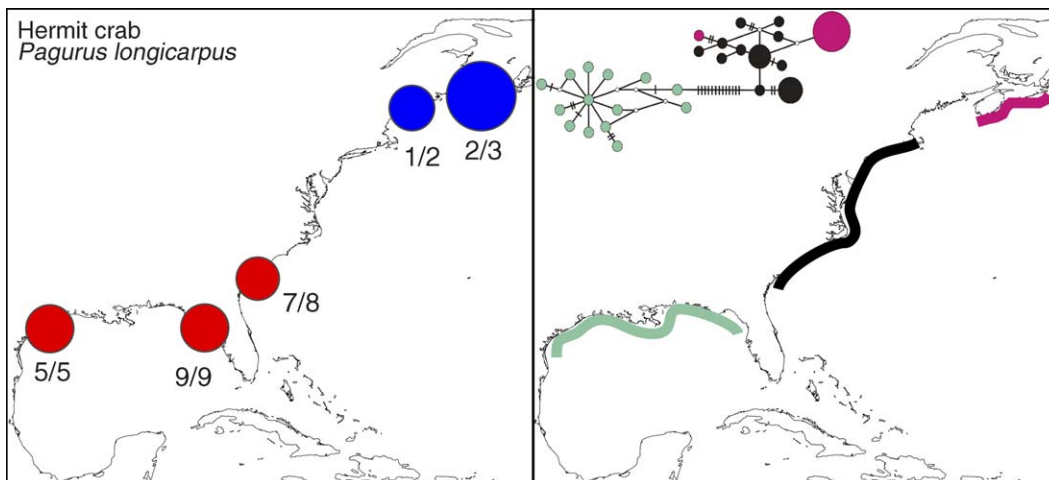

FIG. 7.   *Pagurus longicarpus* (hermit crab) in northwest Atlantic, based on COI sequences from Young et al. (2002). See Fig. 4 legend for explanation of bubble plots and colors.
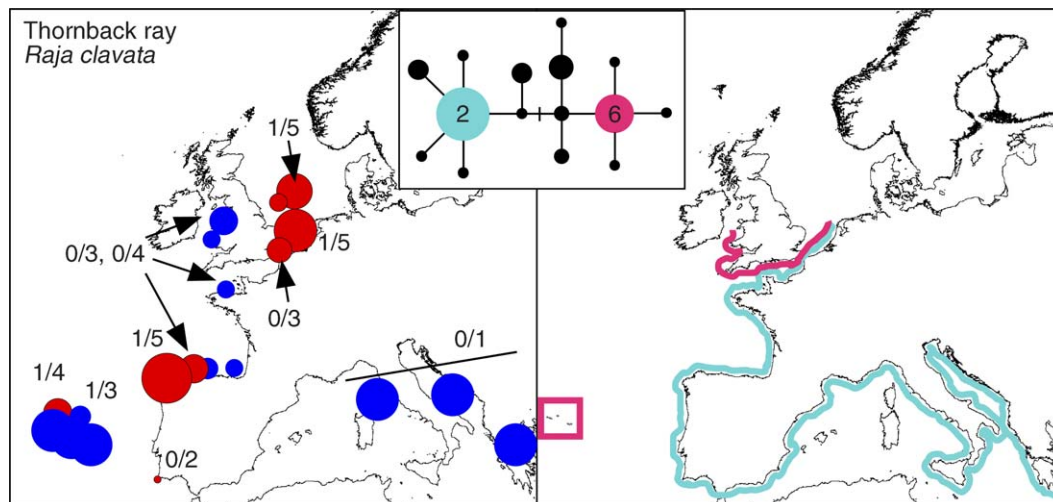
Fig. 8. *Raja clavata* (thornback ray), based on cytochrome *b* data from Chevolot et al. (2006). See Fig. 4 legend for explanation of bubble plots and colors.

ical concordance with the genealogy (Fig. 7), so this is an example of Model IIIB.

Model IV. Two or more refugia-derived (conspecific) populations with secondary contact

Raja clavata (the thornback ray) shows two areas of high genetic diversity (eastern English Channel to Southern North Sea and northwest Iberia) and low diversity elsewhere (Fig. 8). Chevolot et al. (2006) present evidence that population expansion greatly pre-dated the LGM, which affected the distribution rather than the size of populations. The haplotype network and distributions (Fig. 8; Chevolot et al. 2006), which correspond to Model IVB, point to the Azores as one refugium. In the Azores, despite the low diversity the common haplotype (6) is an ancestral one and mutations have given rise to descendant haplotypes, three of which are restricted to the Azores. A private haplotype in northwest Iberia suggests this was a second refugium. The single central haplotype (2) widely dispersed in the Mediterranean and Black Sea could imply that the Mediterranean was a refugium that has subsequently undergone an extreme population bottleneck. However, this result also resembles the expectations of rapid range expansion from Iberia. The high diversity in the English Channel/North Sea area (with 5 haplotypes in both areas; see Fig. 8) is interpreted as the result of admixture of colonists from the Azores refugium, an Iberian refugium, and possibly also a boreal refugium (the putative origin of one private allele found in the North Sea; Fig. 8).

The key genetic signature for this model is that the secondary contact zone will show an increase in diversity as the recolonists from the two refugia come into contact. This result must however be distinguished carefully from that of secondary contact due to non-refugial events like anthropogenic admixture, or long-

diverged cryptic species coming into sympatry, such as the narrow parapatric boundary at the tip of Brittany seen for two clades of the polychaete worm *Pectinaria koreni* (Jolly et al. 2005, 2006; Fig. 9; see also Plate 1). While clade 2 has a southern distribution and shows a signature of northward range expansion after the LGM, clade 1 has a bimodal haplotype network with the two most common ancestral haplotypes 4 and 10 distributed throughout the area as a result of post-vicariant admixture, but probably at a time pre-dating the LGM. Two central and high frequency haplotypes, however, are geographically restricted. The first is found in the eastern English Channel–southern North Sea (haplotype 14), and the second in the Irish Sea (haplotype 60), as a result of post-glacial colonization of these areas from two northerly refugia that have not yet been located.

The gastropod *Cyclope neritea* (see Plate 1) exemplifies an artificially accelerated admixture process, due to its association with cultivated oysters. High genetic diversity is seen in three oyster cultivation areas (Bay of Biscay and two Mediterranean regions) and low diversity was found in northern Iberia (the natural northern limit of this species, gradually extending northward), southern Iberia, and most of the Mediterranean (Fig. 10; Simon-Bouhet et al. 2006). The simplified network and distributions of three major clades show artificial admixture resulting from multiple introductions into the Bay of Biscay from different Mediterranean sources. The proportion of private alleles in the native populations is low because they are shared with the introduced populations. A high proportion of private haplotypes at a site in south Brittany (Fig. 10; S) was linked to frequent aquaculture exchanges with Venice Lagoon (Simon-Bouhet et al. 2006) but also suggests admixture from another, non-sampled native area.
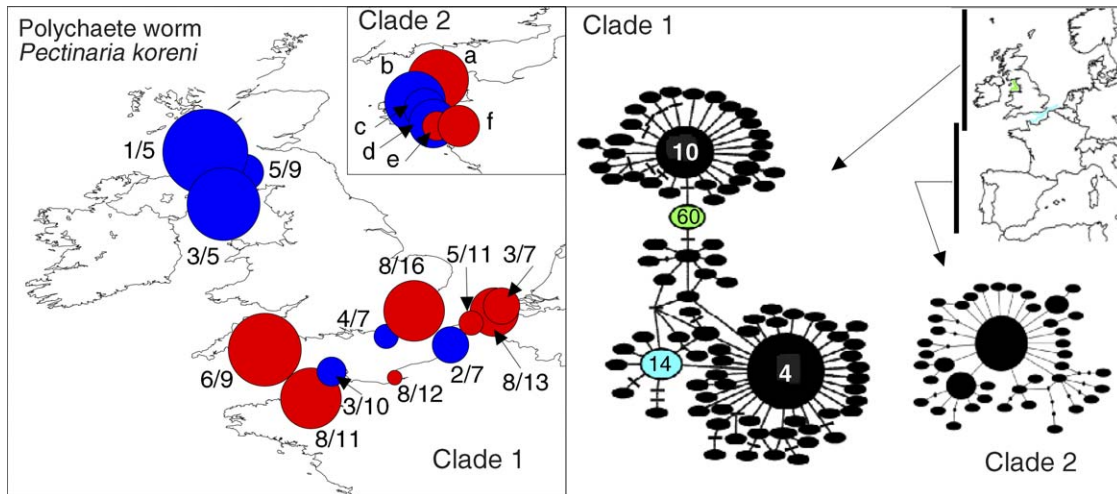
Fig. 9.   Polychaete worm *Pectinaria koreni*: two clades recognized as sibling species, based on analyses of COI sequences in Jolly et al. (2005, 2006). For clade 2 populations, proportions of private haplotypes are: a, 5/7; b, 3/5; c, 10/12; d, 2/4; e, 8/10; f, 4/5. See Fig. 4 legend for explanation of bubble plots and colors.

Are terrestrial models of post-glacial signatures adequate for marine environments?

We have found evidence for marine species that resembles terrestrial scenarios of leading edge colonization processes producing large areas of genetic homogeneity and more southerly populations (trailing edge) with a higher proportion of unique, localized haplotypes. One difference from most terrestrial observations is that where we have evidence of both a southern refugium and a periglacial refugium (e.g., for *Fucus serratus*) the southern refuge has lower genetic diversity. We show here that even in the marine environment genetic signatures of refugia can be distinguished from those of other processes e.g., secondary contact from non-refugial sources. Examining detailed distributions of haplotypes with known relationships can provide evidence of the locations of marine glacial refugia despite the high potential for large-scale dispersal for many marine species. In several cases, ancestral haplotypes are seen to have recently and rapidly colonized newly available areas such as the North Sea.

We have shown clearly that haplotype diversity, taken alone, provides misleading inferences for glacial refugia, even when diversity is shown on maps on a species-by-species basis as here rather than by plotting of indices of latitudinal genetic diversity. The highest diversity, as
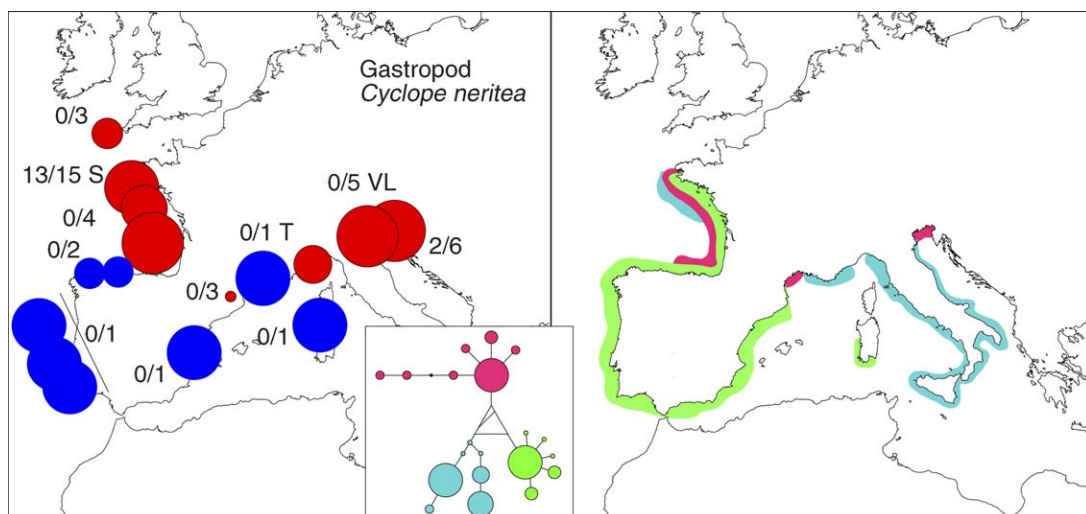

Fig. 10.   Gastropod *Cyclope neritea*, based on analyses of COI sequences in Simon-Bouhet et al. (2006): simplified haplotype network showing three major clades and map with distribution of clades showing multiple introductions into the Bay of Biscay from different Mediterranean sources (VL, Venice Lagoon; T, Thau Lagoon; S, Séné). See Fig. 4 legend for explanation of bubble plots and colors.

found by Petit et al. (2003), is in areas where emigrants from multiple putative refugia come into secondary contact. The English Channel has particularly high genetic diversity for several species, with various probable causes inferred from details of the haplotype distributions and relationships. For some species (e.g., *Raja clavata*), this is seen to be due to admixture from different refugia, whereas for the two model seaweeds a refugium near this area is postulated. In addition, multiple other factors including contemporary differential selective processes operate here to enhance diversity (Jolly et al. 2005).

Despite a good match between the expected genetic consequences of glacial refugia and our observations for a range of marine species, some caveats are necessary. Firstly, the precise locations of the actual refugia are not necessarily associated with these genetic signatures. For example, we have discussed a possible refugium in the vicinity of the English Channel's Hurd Deep, but acknowledge that only direct evidence from sediment coring could provide unequivocal evidence. The most significant genetic test of a refugium is evidence of long population persistence in the form of rigorous multilocus estimates of divergence time. However, these estimates depend on molecular clocks, which are fraught with difficulties including the requirement for calibration points within the timeframe of interest due to variation over time in divergence rates (Audzijonyte and Väinölä 2006, Ho et al. 2007). Furthermore, increasing success with ancient DNA is revealing conflicts between phylogeographic inference from modern sequences and the results from sequencing old samples (e.g., the brown bear [Valdiosera et al. 2007]).

Moving forward

It is apparent that a consistent sampling scheme for multiple species along all coasts of the North Atlantic would be of great benefit for comparative inference of post-LGM migration pathways. At present, it is still very hard to find even a few locations that have been sampled for a range of appropriate species, which poses a real problem for conducting meta-analyses among marine biota, especially if they are to be based on non-commercial and non-invasive biological species with similar life-history characteristics.

One of the challenges in phylogeography is to quantitatively assess the spatial and temporal attributes of populations, particularly when these have repeatedly contracted and expanded from refugia (Jesus et al. 2006), and new methods are being developed for this (Hickerson and Cunningham 2005, Hickerson et al. 2006). Future research needs to discriminate admixture from ancestral polymorphism. Promising approaches include Bayesian identification of admixture events using multilocus molecular markers to separate the ancestral sources of the alleles observed in different individuals (Corander and Marttinen 2006), and "power analysis" with simulation of a variety of historical

PLATE 1. (Upper and center photos) The introduced gastropod *Cyclope neritea* has been spread anthropogenically in Europe by aquaculture activities such as this oyster farm in Alvor, Portugal. Here, the gastropods are consuming a dead fish. Photo credits: Benoit Simon-Bouhet. (Lower photo) The polychaete *Pectinaria koreni* (Malmgren) out of its tube. The diameter of the cephalic disc (the head) is ~5 mm. The species consists of two cryptic species with different geographical distributions. Photo credit: M. T. Jolly.

scenarios to see whether any can be excluded. Deciphering migration-drift and selection effects could perhaps be achieved with model species (fish or bivalves) for which numerous genes have been analyzed in genomic projects. Finally, ecological niche modeling is proving to be a valuable adjunct to genetic studies, helping to hindcast population levels by identifying suitable habitat during range contractions (Bigg et al. 2008, Dépraz et al. 2008), and confirming the success of phylogeographic analyses in identifying refugia (Waltari et al. 2007).

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