Rita Castilho

Sumário pormenorizado da lição

[Sumário pormenorizado da lição apresentado nos termos da alínea c) iii) do n.º 3 do artigo 4.º do Regulamento de atribuição do título académico de agregado da Universidade do Algarve, publicado no Diário da República, 2.ª série, n.º 33, de 17 de fevereiro de 2020]

Marine Phylogeography of Northeastern Atlantic and Mediterranean

Patterns and Processes

Sumário Pormenorizado da Lição

Rita Castilho

[Sumário aula apresentado nos termos da alínea c) iii) do n.º 3 do artigo 4.º do Regulamento de atribuição do título académico de agregado da Universidade do Algarve, publicado no Diário da República, 2.ª série, n.º 33, de 17 de fevereiro de 2020]

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1. Preface

This document is the lesson summary of the candidacy to “Agregação” under the terms of Decree-Law nº 239/2007 of 19 June of the Ministry of Science, Technology and Higher Education. I have requested permission to present the documents in English because this lesson is part of the course plan for the (international) Master's Degree in Marine Biology at the University of Algarve, which is entirely taught in English. The student’s background is varied, from Environmental Sciences, Life Sciences, Biology, and Marine Biology. Therefore, the lesson is an overview covering basic phylogeography concepts illustrated with examples. A master of science student should be able to follow the lesson and to dig in afterwards during the individual study in aspects that are more unfamiliar.

The lesson is a general review of the Northeastern Atlantic and Mediterranean phylogeography, mainly focused on fish, although other biological models are also referred to when appropriate. We will first build background information before addressing the case studies. As background information, we will refer to (1) the formation of the Atlantic and Mediterranean basins; (2) the significant changes in their conformation, (3) the geological and climatic events and their impacts on marine populations; (4) the main drivers of marine dispersion and differentiation; (5) the genetic assessment of connectivity among populations; (6) the main molecular markers used in phylogeography. With this foundation laid out, we proceed to expose various illustrative case studies that represent examples that follow hypotheses and expectations and others that are exceptions, showing the richness of knowledge that can be derived from phylogeographic studies.

Most of the MBM selected students are from Environmental Sciences, Life Sciences and Biology or Marine Biology. To these students, all the class content is intellectually accessible, and students are given enough supporting documentation to take up during the individual study. Over the years, there was no need to make specific adjustments to cater to eventual students’ background deficiencies.

The students intended learning outcome for this lesson is to understand what is the study of the phylogeography of marine organisms and its relevance to the evolutionary history, distribution patterns, and speciation patterns of marine species. Marine phylogeography, besides being an intellectually interesting and motivating discipline, has also numerous applications, including:

Conservation: can provide valuable information about the genetic diversity of marine populations, which is important for the conservation and management of endangered species.

Evolutionary history: by studying the genetic variation of marine species, researchers can reconstruct their evolutionary history, including their origins, dispersal patterns, and diversification.

Biogeography: can contribute to understanding the spatial and temporal patterns of biodiversity in the ocean, and how these patterns are influenced by factors such as ocean currents, historical climate change, and geography.

Fisheries management: the genetic structure and connectivity of marine populations is critical for managing fisheries sustainably and preventing overfishing.

Aquaculture: can inform the development of aquaculture practices by identifying genetic differences among populations that could affect their adaptability to aquaculture conditions.

2. Introduction

Phylogeography studies the geographical distribution of genetic variation within and among species. Molecular biogeography can be summarised under the designation of “phylogeography”, a term coined almost forty years ago (Avise et al., 1987). It describes the branch of science that explores and interprets the geographical distribution of genealogical lineages. We can imagine phylogeography as the result of overlapping two information layers containing geographical data and genetic data with major questions such as what is the number of lineages within a species and their geographical distribution, or is the genetic diversity of a species higher in the glacial refugia (Table 1).
Based on a sample of individuals and genes, phylogeographers can propose biogeographic hypotheses deciphering the evolutionary pathways of population-level reproductive isolation and their origin and dispersal mechanisms. By providing the ancestral structure of populations influenced by population history, phylogeographers can use current genetic data to understand the physical and biological milieu of populations prevalent at the time. Temporal variations can also be predicted. To such end, phylogeography has made significant contributions in many areas of biology and earth sciences, speciation, historical biogeography, human evolution, biodiversity studies

Table 1. Chart summarizing research questions, time frames, and processes addressed in single-taxon and comparative phylogeographic studies (adapted from Marske et al., 2013).

Study type	Single taxon	Multiple taxa (comparative)
Study approaches and major questions	Discovery of, or Testing hypotheses about, diversity, divergence, and range dynamics: • Single lineage or several cryptic lineages? • Congruent patterns across co-distributed taxa? • Dispersal or vicariance as mechanisms of lineage diversification? • Into and out of glacial-age refugia? • Responses to geological events in Earth history?	
Phylogeographic Timeframes	Quaternary (2.58 million years ago – today)	Neogene (23.03 - 2.58 Mya)
Temporally structured phylogeographic processes	Late Quaternary range dynamics (range expansion, range contraction, range shifting)	Quaternary / Neogene lineage divergence development of 2 or more eographically-structured 'phylogroups' within any single taxon)
Drivers of phylogeographic processes	glacial / interglacial cycles creating then dissolving one or more refugia as climates change (cycles of range contraction and range expansion)	multiple glacial refugia geological events that create opportunities for divergence, e.g., across dispersal barriers, or on emerging landscapes
Consequences of phylogeographic processes	Including: • Shifts in population genetic structure and diversity • Speciation • Adaptation and Niche evolution	

and taxonomy (e.g., Ayre et al., 2009, Crandall et al., 2019, Marko et al., 2010, Patarnello et al., 2007).

There are many regions of interest in marine phylogeography throughout the world. Among them, the Caribbean-Pacific region divided by the Isthmus of Panama; the Gulf of Carpentaria, a large, shallow sea enclosed on three sides; the Indonesian seaway; or the Atlantic-Mediterranean divide, and the Northeastern-Atlantic region whose shores were greatly impacted by the last glaciation. Marine coastal systems in the Northern Hemisphere have survived a dynamic history of climatic fluctuations. Cycles of Pleistocene glaciations over the past 2.6 million years are widely accepted to have left a significant imprint on the genetics of marine coastal populations, with refugia scattered throughout the Atlantic providing periodic sanctuary from the expansion and contraction of ice sheets (Maggs et al., 2008).

In the case of northeastern Atlantic fish, phylogeography can help identify patterns in the distribution of genetic variation within and among different fish species in this region and the processes that have shaped these patterns. Several factors can influence the phylogeography of northeastern Atlantic fish, such as the region's physical geography, the main currents and proximity to the Mediterranean and the historical events that have occurred in the region. For example, the retreat of glaciers at the end of the last ice age may have significantly impacted the distribution of certain fish species in the region. Overall, the phylogeography of northeastern Atlantic fish can provide insights into these species' evolutionary history and current population dynamics and the environmental and historical factors that have shaped their distribution.

2.1. The Northeastern Atlantic

The Atlantic is a smaller basin than the North Pacific Ocean and presents an inverted U-shaped coastline with Greenland, Iceland and the Faroe Islands in the middle of the northern part of the ocean. The Atlantic Ocean is one of the world's largest and oldest oceans, and it has a complex and fascinating history. The Atlantic Ocean formed around 200 million years ago (Mya) during the breakup of the supercontinent Pangaea. Pangaea was a supercontinent that formed around 300 Mya when all of the Earth's land masses were joined together in a single landmass. Around 200 Mya, Pangaea began to break apart due to movements in the Earth's crust. This process took millions of years and eventually resulted in the formation of the Atlantic Ocean. The process of the Atlantic Ocean's formation is still ongoing, and it is driven by plate tectonics. The Earth's crust is formed by several large tectonic plates that move slowly over time due to convection currents in the mantle. The Atlantic Ocean is formed by the separation of two of these plates, the African Plate and the South American Plate.

As these plates move apart, magma from the Earth's mantle wells up to fill the gap, forming a new oceanic crust. This process creates a mid-ocean ridge, which is a mountain range that runs down the centre of the Atlantic Ocean. The Atlantic Ocean is still widening today at a rate of about 2.5 centimetres per year. The Northeastern Atlantic is a large region of the Atlantic Ocean located off the northeastern coast of Europe, extending from the Arctic Circle in the north to the Iberian Peninsula in the south (Figure 1). Due to the Atlantic history, fish, invertebrate, and seaweed populations show high levels of genetic differentiation in their Northern part (e.g. Bringloe et al., 2022, Maggs et al., 2008, Robalo et al., 2020). Conspecific populations on both sides of the North Atlantic were isolated during the Ice Age, and some taxa were eradicated from the Northern Atlantic and re-established after the Last Glacial Maximum (18-20 Mya). Moreover, adding complexity, some populations in the Northwest Atlantic share a closer genetic relationship with the North Pacific than with the Northeast Atlantic (e.g., seagrasses and sea urchins) (Olsen et al., 2004).

The Northeastern Atlantic includes the North, Baltic, and Atlantic Ocean waters surrounding the British Isles, Ireland, and the Scandinavian Peninsula. This region is home to diverse fish species, including cold-water species such as cod, haddock, and herring, and warm-water species such as mackerel, sardines, and tuna. It is also an important region for commercial and recreational fishing, with many countries in the region relying on these activities as a significant source of income and food. Importantly, this area is also home to several important marine ecosystems, including estuaries, tidal flats, and offshore banks, which provide habitat for a wide range of species and support important ecological functions such as nutrient cycling and carbon sequestration.

The Baltic, North Sea, and Mediterranean biogeographic regions are somewhat isolated from the Atlantic Ocean by narrow straits that often coincide with phylogeographic transitions (Johannesson and André, 2006; Patarnello et al., 2007). But to each example, it is possible to find exceptions; for instance, in some Sparid species, some display marked genetic divergence across one phylogeographic barrier while others show little intraspecific differentiation across the very same barrier (Bargelloni et al., 2005).

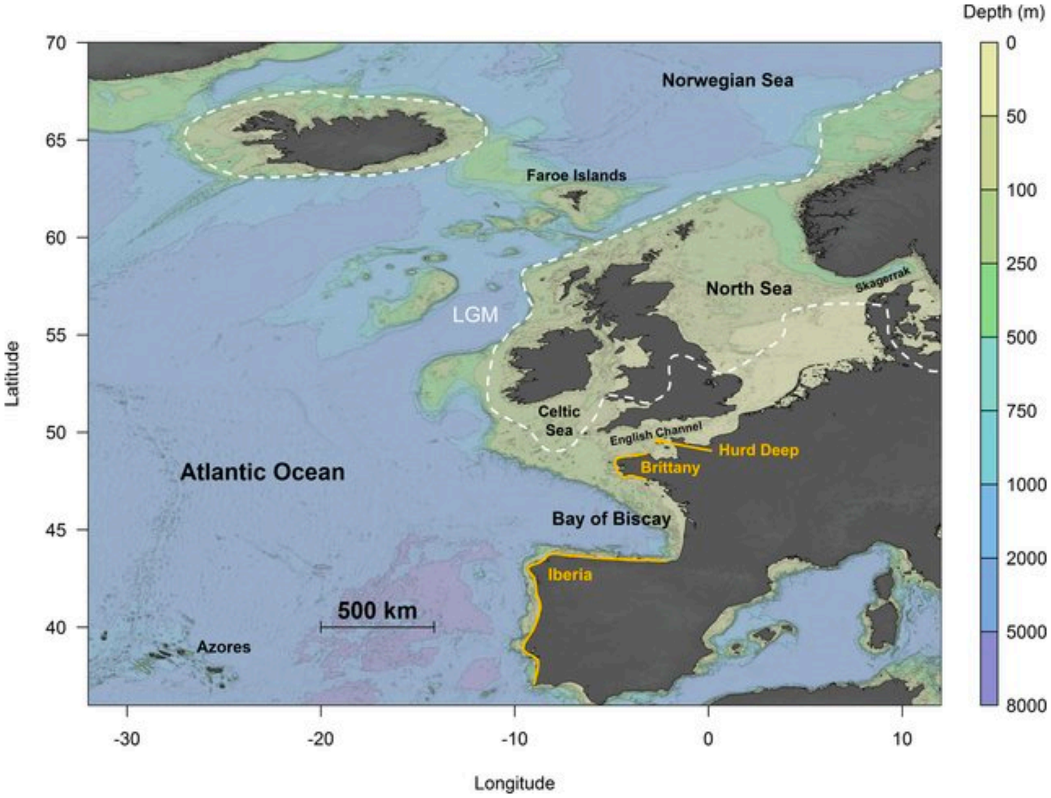


Figure 1. Topographical map of the northeast Atlantic Ocean. The white dotted lines represent the maximum extent of ice cover during the Last Glacial Maximum (LGM) (redrawn from Hughes et al., 2016). Orange lines indicate putative refugia: Hurd Deep (the deepest point in the English Channel), Brittany and Iberia (from Jenkins et al., 2018).

2.2. The Mediterranean Sea

The Mediterranean Sea is a large body of water that lies between Europe, Africa, and the Middle East. It has a rich and varied history, and its formation is closely tied to the region's geology. The Mediterranean Sea has several smaller bodies of water within its basin, including the Adriatic Sea, the Aegean Sea, the Alboran Sea, the Balearic Sea, the Ligurian Sea, the Tyrrhenian Sea, and the Ionian Sea. The Alboran Sea is a small sea located in the western Mediterranean Sea, between the Iberian Peninsula and the north of Africa. It borders Spain to the west, Morocco to the south, and Algeria to the east (Figure 2).

Figure 2. The Mediterranean Sea and its constituent seas.
Modified from source: https://news.mit.edu/2020/why-mediterranean-climate-change-hotspot-0617

It is not uncommon for there to be multiple gyres in a single ocean or sea. In the case of the Alboran Sea, there are two main gyres: the Almeria gyre and the Oran gyre (Figure 3). These gyres are named after the cities of Almeria and Oran, which are located on the coasts of Spain and Algeria, respectively. The Almeria gyre is a large clockwise-rotating current system that is located to the west of the Almeria-Oran front. The Oran gyre is a smaller counterclockwise-rotating current system located to the east of the front. Both of these gyres play important roles in the water circulation in the western Mediterranean Sea, frequently acting as a phylogeographic barrier (Galarza et al., 2009).

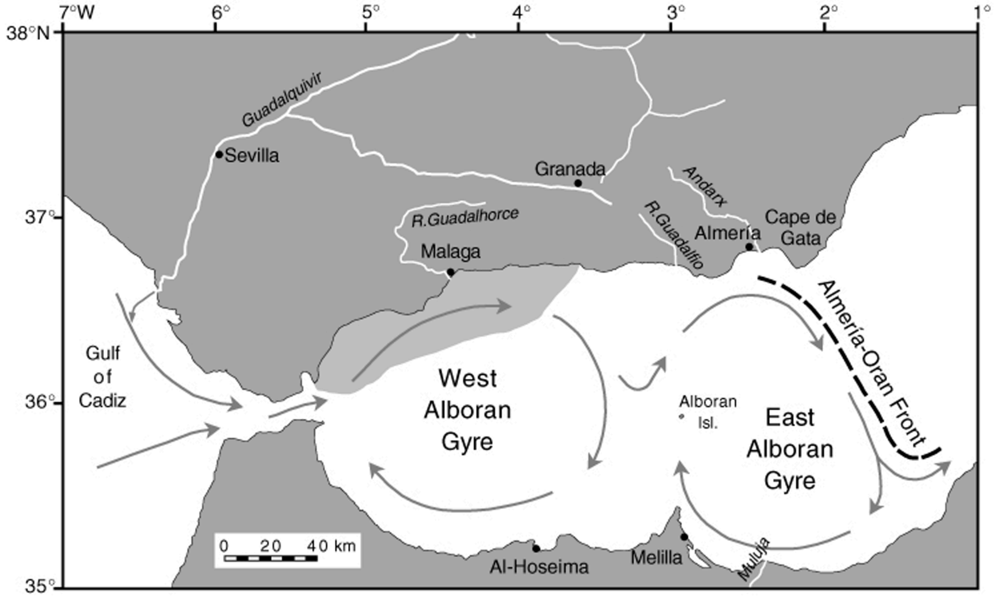


Figure 3. Outline of the major oceanographyc features in the Alboran Sea, highlighting the two anticyclonic eddies formed by the entering Atlantic waters. Source: www.eoearth.org/view/article/149961

Today, the Mediterranean Sea is a smaller version of the Tethys Sea. The Tethys Sea was a large body of water that existed during the Mesozoic era (252 to 66 million years ago) and was located in the region that is now the Mediterranean Sea. The Tethys Sea eventually closed and was replaced by the Mediterranean Sea, as the African and Eurasian plates collided and the region was uplifted, forming the Alps and other mountain ranges. Later, during the Late Miocene epoch is known as the Messinian Salinity Crisis (MSC; 6.14–5.96 Mya) (Rouchy and Caruso, 2006), the Mediterranean Sea was cut off from the Atlantic Ocean by tectonic activity that closed the Strait of Gibraltar (Figure 4). As a result, the Mediterranean Sea became a closed basin, and the evaporation of its water exceeded the rate at which rivers and other sources replenished it. This led to a dramatic increase in salt concentration in the Mediterranean Sea, and eventually, the sea became almost completely dry (Hsü et al., 1973). The Messinian salinity crisis significantly impacted the region's geology and climate. The dry conditions led to the formation of large salt deposits, which are still present in the region today. The crisis also had a major impact on marine life in the region, as many species could not survive the elevated salinity and dry conditions. After several thousand years, the Strait of Gibraltar reopened, and the Mediterranean Sea was once again connected to the Atlantic Ocean. The influx of freshwater from the Atlantic Ocean helped dilute the high salt levels in the Mediterranean Sea, and the sea gradually refilled over time. The Mediterranean Sea has changed significantly over time and is still influenced by tectonic activity today. The region is home to several active fault lines, and earthquakes are common. The Mediterranean Sea is also slowly closing as the African Plate slowly moves northward and collides with the Eurasian Plate. This process is causing the Mediterranean Sea to become smaller over time.

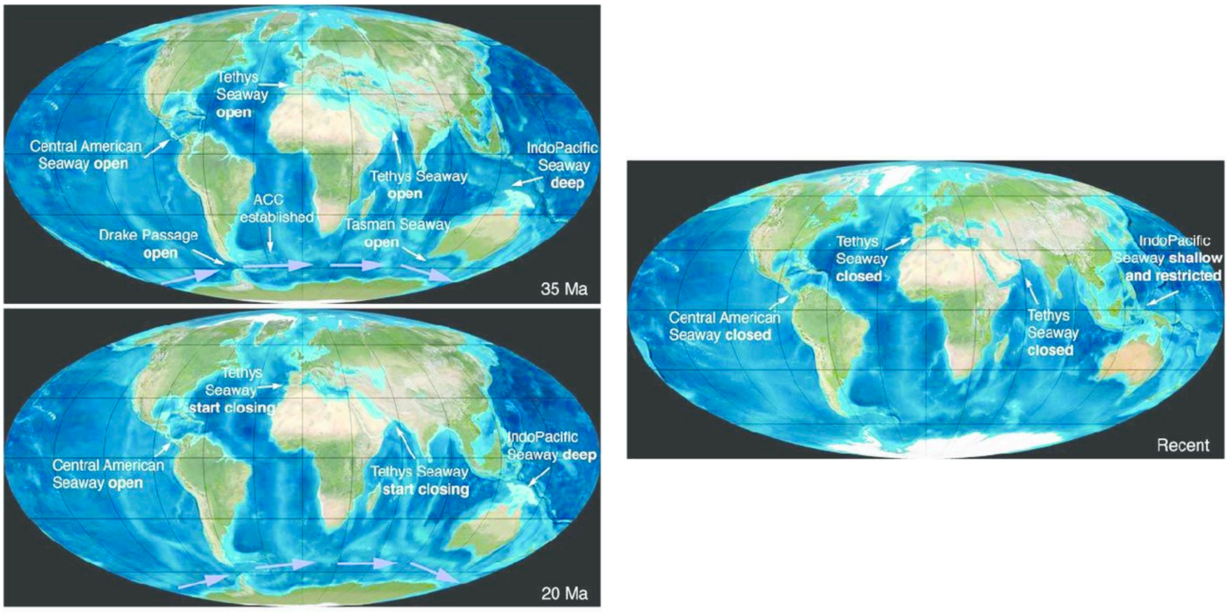


Figure 4. Coastline maps indicating the timing of opening and closure of the Tethys Sea. At 35 million years ago the Tethys Seaway was still open, but ca. 18-20 Ma it closed its connection to the Indian Ocean, at the east, and later, at million years ago Ma, closed the connection with the Atlantic Ocean.

The Atlantic Ocean and the Mediterranean Sea are connected by the Strait of Gibraltar, a narrow channel between the southern tip of Spain and northern Morocco that opened 5.3 Mya. The Strait of Gibraltar is about 14 kilometres wide at its narrowest point, and it is an important transportation route for ships travelling between the Atlantic Ocean and the Mediterranean Sea. Overall, the connection between the Atlantic Ocean and the Mediterranean Sea is an important aspect of the geology and climate of the region, and it has significant ecological and economic importance. The limited dispersal between the Mediterranean and the Atlantic constitutes an important natural laboratory to study the diversifying impact on marine life, separating two distinct ecosystems. Marine biologists who study the Mediterranean-Atlantic divide are interested in understanding how the divide affects the distribution and dispersion of marine species and the interactions between individuals of the same on either side of the divide. From the biological and genetic viewpoint, the exact location of the separation between the Atlantic and the Mediterranean is not universal. One thing, however, seems to be established: however historically, geographically or geopolitically relevant, the straits of Gibraltar bear little influence on restricting the movement of marine organisms. If indeed a boundary or phylogeographic barrier exists for diverse marine species, it is located at the Almeria-Oran front (e.g., *Engraulis encrasicolus*, Silva et al., 2014) inside the Western Mediterranean. However, this barrier is not universal, and for many other species, there is no detectable sign of any division (e.g., *Scorpaena maderensis*, Francisco et al., 2021). We will see this in more detailed examples in the study-case section.

2.3. Geological and climatic events and their impact on marine populations

Many geological events have influenced climate throughout the Earth's history and promoted dramatic fluctuations: (1) volcanic eruptions lead to short-term cooling effects on the climate but also to long-term warming when the gases released by the eruption trap heat in the atmosphere; (2) changes in the Earth's orbit affect the amount of solar radiation reaching Earth's surface, contributing to long-term climate change and (3) changes in the Earth's surface due to processes such as mountain building, erosion, and plate tectonics, which can close or open seaways, are among the most prominent. Plate tectonics have originated the most important marine barriers to gene flow, preventing the movement of individuals between populations. These are called “hard barriers” as they impede the mixing of all marine species across the barrier and originate vicariance events, large-scale events that lead to the separation of populations. These events significantly impact the phylogeography of marine species, as they lead to the separation of populations and the development of genetic differences and divergence between them. Some examples include the formation of land bridges during periods of low sea levels. Land bridges can form between land masses that were previously separated by water, e.g. the formation of Doggerland (Figure 1) and the formation of the Isthmus of Panama or the closure of the Tethys seaway (Figure 5, barrier 7). In the region of interest of this lesson, there are several identified phylogeographic breaks and barriers to dispersion (Figure 5). Most are called “soft barriers”, that is porous barriers for some

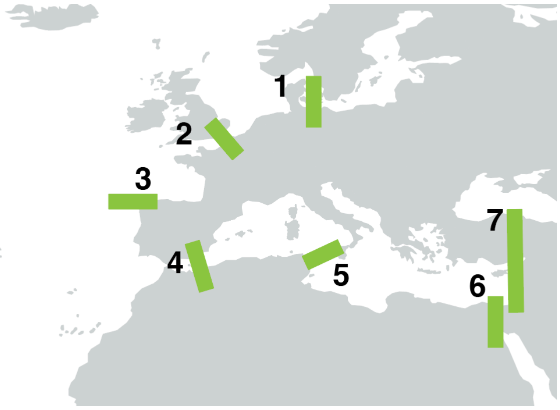


Figure 5. Phylogeographic breaks and barriers to dispersal in the Atlantic Ocean and Mediterranean sea. (1) Baltic Sea - North Sea, (2) English Channel - North Sea, (3) Bay of Biscay - Lusitania, (4) Almeria-Oran front, (5) Siculo-Tunisian strait, (6) Suez Canal and (7) Tethys land bridge.

species but not all species. Those are barriers enforced by currents, temperature or salinity, for instance.

Of all the changes that occurred in the past, the glaciations of the Quaternary led to abrupt changes in sea level of ca. 130m below the present-day level (Yokoyama et al., 2000) and consequently to changes in the distribution of many marine species, particularly in the Northern Hemisphere, where these glaciations cycles had more impact (Figure 6).

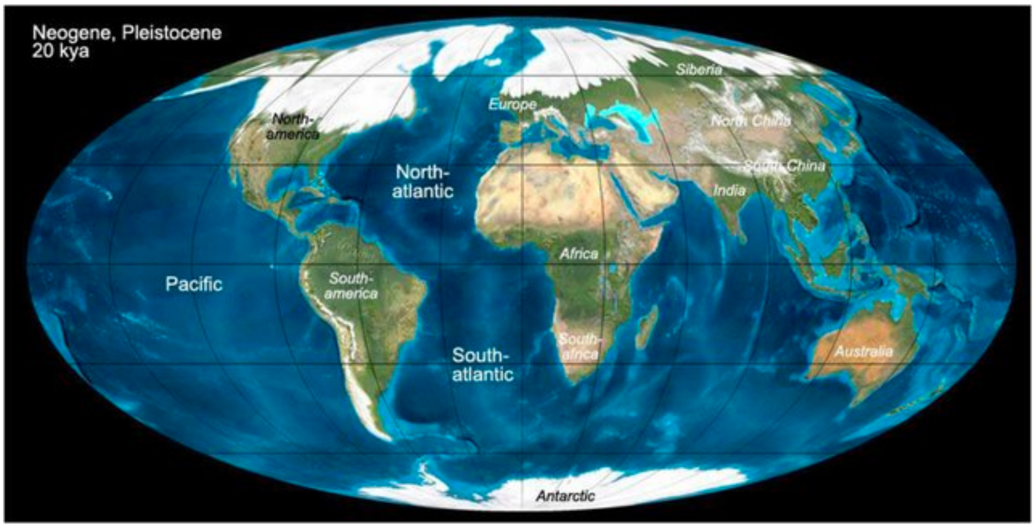


Figure 6. Land masses during the last glaciation (http://www.kerbtier.de/Pages/Themenseiten/enPhylogenie.html).

Phylogeographic surveys suggest that large-scale patterns of population genetic diversity are often shaped by climate-determined range dynamics (Hewitt 2000, 2004) rather than the genetic randomness of populations. Quaternary climatic fluctuations greatly affected species' geographic ranges and demography, particularly at high latitudes (Hewitt, 2004; Maggs et al., 2008). The expansion-contraction model (Provan & Bennett, 2008; Marko et al., 2010) proposes consecutive cycles of range contractions during the glacial advance and subsequent rapid expansion during interglacial periods. The expansion-contraction model corresponds to geographic range shifts, that is, changes in the distribution of marine species over time. As a result of range shifts, some species may adapt to the changing conditions, while others may struggle and even become extirpated or extinct.

The expansion-contraction cycles have strongly influenced the distribution of genetic variation in high-latitude organisms and can now be detected using molecular tools and phylogeographic analyses (Maggs et al., 2008; Marko et al., 2010; Jenkins et al., 2018) (Figure 7). Certain areas identified as glacial refugia were more genetically diverse (Maggs et al., 2008), prompting several studies to assess the glacial consequences in populations' genetic diversity, which can still be traced back today (Jenkins et al., 2018; Maggs et al., 2008).

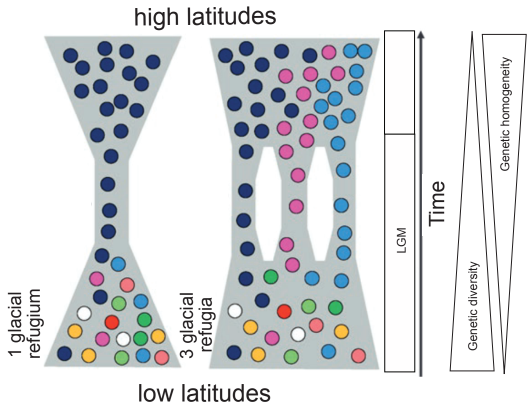


Figure 7. Two possible glacial refugia scenarios and respective genetic consequences during glacial periods (i.e., LGM) and postglacial range expansion after ice retreat, leading to genetic bottlenecks in current distribution of species. Species ranges are drawn in gray; each circle represents an individual; different colors represent genetic variation (e.g., different haplotypes). Modified from Guillemin et al.

The observation of the poleward decrease of genetic diversity within and among populations prompted the proposal of the 'leading edge' colonisation model. In this model, range expansions involve mostly populations from the colonisation front and are largely controlled by rare long-distance dispersal events followed by exponential population growth (Figure 8). Therefore, marginal populations at the rear edge rather than core populations generally have the highest proportion of species genetic diversity (e.g., Petit et al., 2003; Hewitt, 2004).

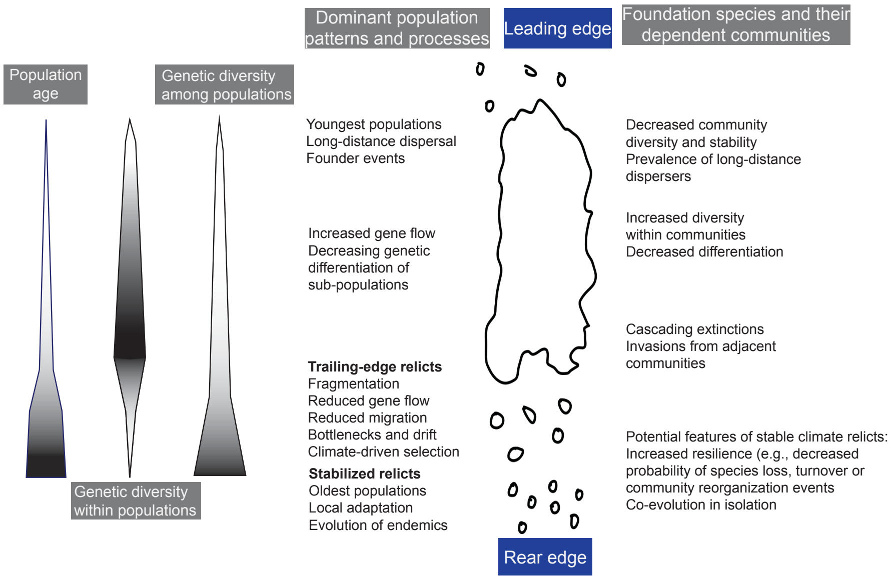


Figure 8. Genetic and demographic consequences of populations, communities and ecosystem arising from the poleward range expansions. The width of the bars on the left is a quantitative representation of age and genetic diversity within and among populations. The text on either side of the range map summarizes the likely dominant population (left) and community and ecosystem (right) responses to changing climate (modified from Woolbright et al., 2014).

Geographical changes are well documented around the range edges (Sunday et al., 2012) (Figure 9). Environmental temperature largely drives these changes (Poloczanska et al., 2013). For example, when a species' cold tolerance limits the range at its edges, warming is expected to improve individual performance, such as activity, growth, immune response, survival and fertility (Portner and Farrell, 2008), leading to population growth. Colder temperatures can drive non-sessile species to lower warmer latitudes with compatible physiological thresholds. When temperatures rise globally, the situation is inverted, and many species will re-colonise higher latitudes that have now compatible temperatures as the ice retreats.

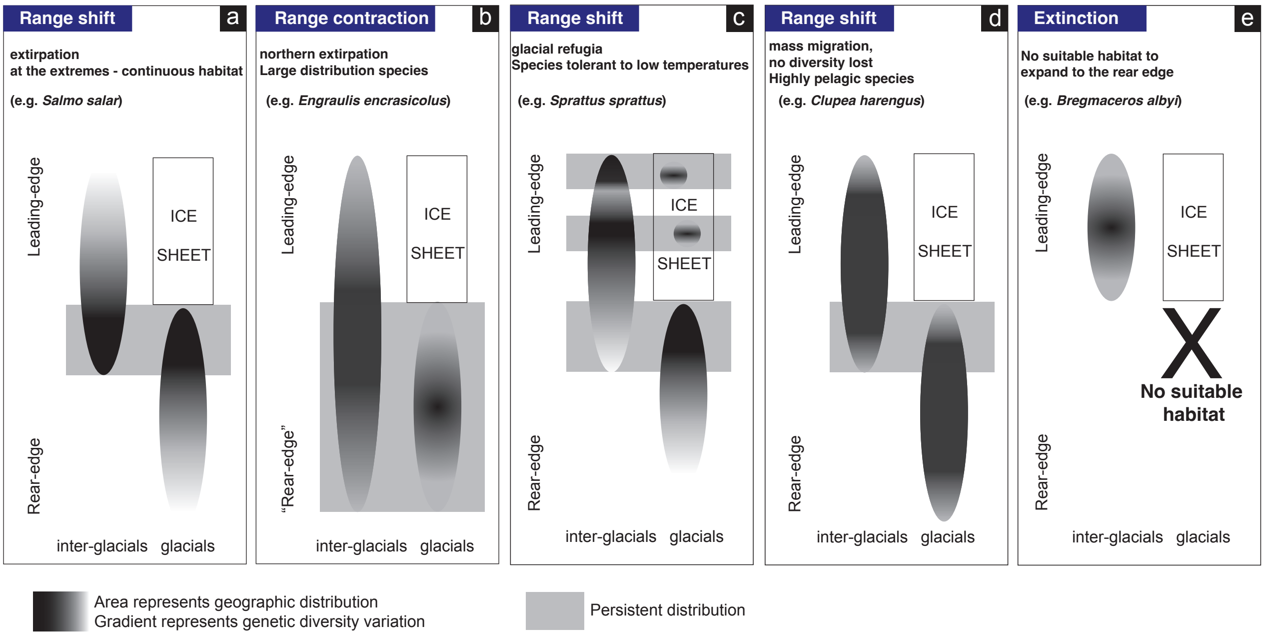


Figure 9. Examples of range shifts in several marine species. Range shifts of temperate marine organisms imposed by glacial and interglacial periods in the Pleistocene; a) species able to track suitable habitats and genetic diversity was maintained were distribution was persistent both in glacial and inter-glacial periods; b) species whose distribution range contracted at the leading-edge and genetic diversity was maintained were distribution was persistent both in glacial and inter-glacial periods; c) species able to track suitable habitats and genetic diversity was maintained were distribution was persistent both in glacial and inter-glacial periods, including refugia within the glacial ice-sheet; d) species with large population sizes and high dispersal ability were capable to move massively and thus preserving diversity; e) species that were not able to survive to Pleistocene shifts and became extinct. Modified from Silva (2014).

Overall, vicariant and non-vicariant events can significantly impact marine species' phylogeography by separating populations and promoting the consequent genetic divergence. Studying the consequences of these past events provides insights into the evolutionary history and population dynamics of marine species, as well as the environmental and historical factors that have shaped their distribution, but also allows some insight into the near future under climate change.

2.4. Promoters of marine dispersion/differentiation

While there are well-known barriers to the dispersal of marine organisms, there are many opportunities for marine organisms to differentiate without the presence of physical barriers. Regardless of adult habitat or latitude, most bony fish species have pelagic larval stages dispersed by ocean currents and biological processes. Dispersal is a process of pivotal relevance for populations and communities' ecological and evolutionary dynamics because of its multiple repercussions on gene flow and demography. Dispersal includes departure or leaving the natal habitat, displacement or movement, and finally, settlement, establishing a novel breeding habitat, and can occur in different life stages, although the planktonic larval life stages are the most prone to dispersal in marine organisms (Figure 10).

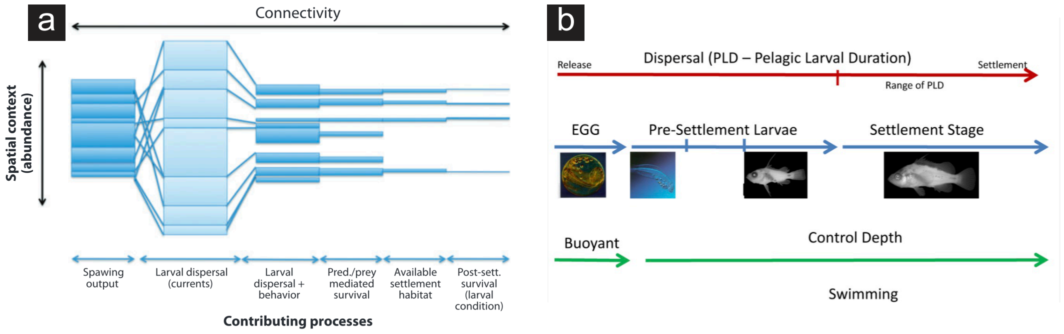


Figure 10. Representation of the processes that act to determine the spatial connections among populations via larval dispersal and survival. (a) from Cowen and Sponaugle (2009), (b) from Leis (2020).

The movement and distribution of marine organisms are promoted by several factors, among which oceanographic currents play a pivotal role. Ocean currents can transport marine organisms long distances during various phases of their development, allowing dispersal to new areas. In its most dramatic way, a tsunami, with extremely powerful waves, can carry along a variety of hitchhikers, such as crustaceans, molluscs, and fish (Carlton et al., 2017). These animals may be swept up by the waves and carried many thousands of kilometres away, colonising other geographic regions where the habitat is suitable.

Larval and adult life characteristics influence connectivity and oceanographic discontinuities between sites. Species with low migratory capacity are more likely to exhibit significant genetic differentiation between regions than species with high migratory capacity, while oceanographic discontinuities reduce gene flow in species with moderate to high dispersal capacities (Pascual et al., 2017) (Table 2).

Table 2. Effect of life history traits on population genetic differentiation and their impact on connectivity reduction mediated by oceanographic discontinuities. From Pascual et al. (2017).

Dispersal capacity	**Low** PLD 1-15 days	**Medium** PLD 16-30 days	**High** PLD > 31 days
Life traits	Habitat formers & benthic vagile	Benthic vagile & limited motility	Pelagic & benthic vagile
Genetic differenciation	**High** High retention rate	**Medium** Intermediate retention rate	**Low** Low retention rate
Effect of barriers	**Low** Weak effect of currents and fronts	**Medium** Intermediate effect of currents and fronts	**High** Strong effect of currents and fronts
Vulnerability	**High** Weak recovery by gene flow from other locations	**Low** Potential recovery by gene flow from other locations	**Low** Potential recovery by gene flow from other locations

2.5. Types of populations

The huge size of the ocean and the small size of most marine reproductive propagules make it difficult to determine whether the propagules colonize far from where they were born or close to their natal origin. Successful dispersers leave a genetic imprint of their movement, providing an indirect way of estimating population connectivity. Here we will briefly describe six patterns repeatedly observed in geographic studies of genetic variation in marine populations: closed, abrupt genetic change, geographic cline, stepping-stone, chaotic genetic patchiness and broad-scale homogeneity (Hellberg et al., 2002).

These different patterns of geographic genetic differentiation (with the exception of the abrupt genetic change population) evolve based on migration rate size (m) and effective population size (Ne) (Figure 11). A population can be completely closed (all recruits from within) if Ne*m is small or completely open (all recruits from other populations) if Ne*m is large. Between these two extremes, we have other (intermediate) patterns. A detailed temporal sampling can show that the seemingly open population is actually composed of a mixed cohort recruited from a relatively small number of breeding adults. Finally, past effects should always be considered, especially for small m and large Ne.

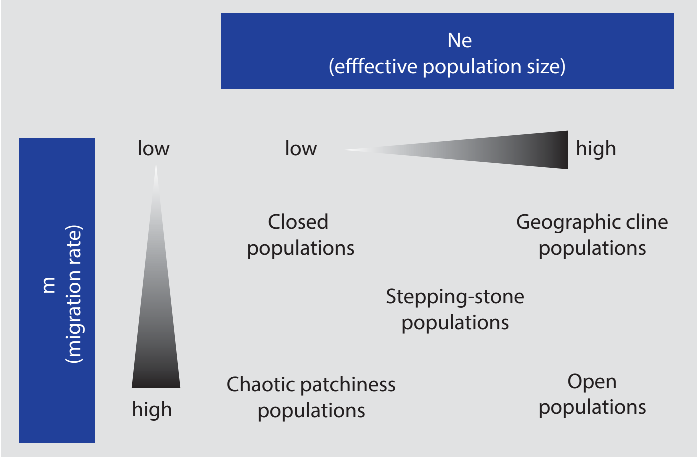
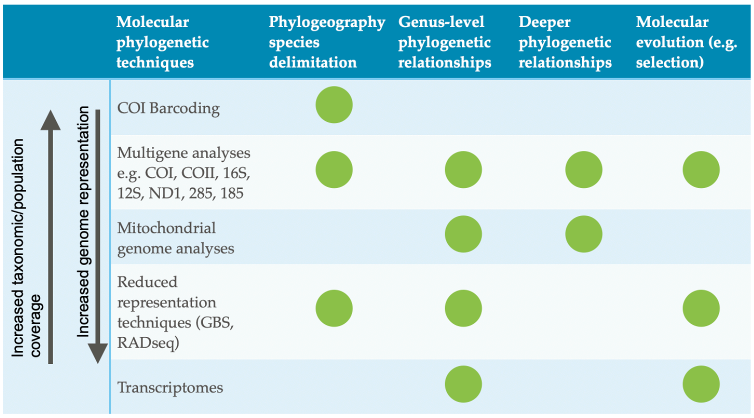


Figure 11. Combinations of migration rate (m) and effective population size (Ne) most favorable to five described patterns (redrawn from Hellberg et al., 2002).

The main genetic feature of *closed populations* is persistent genetic differences from other conspecifics. The extent of this differentiation depends on the combined effects of mutation, drift, migration and selection and their interaction with time and population size. When gene flow ceases between two populations, drift and selection initially play the greatest role in differentiation by acting on existing genetic variation. Over time, mutations lead to the emergence of "private" alleles, i.e., alleles only present in that population.

When there is a *phylogeographic barrier*, a species can suffer a differentiation on either side of the break and produce an abrupt genetic change. Such a pattern suggests that barriers separate species ranges and subsequent gene flow is sufficiently restricted to allow alleles in each population to drift toward alternative allele fixation or reciprocal monophyly.

Table 3. Main molecular phylogenetic techniques available for evolutionary research. Modified from Marchán *et al.* (2022)



Molecular phylogenetic techniques	Phylogeography species delimitation	Genus-level phylogenetic relationships	Deeper phylogenetic relationships	Molecular evolution (e.g. selection)
COI Barcoding	●			
Multigene analyses e.g. COI, COII, 16S, 12S, ND1, 285, 185	●	●	●	●
Mitochondrial genome analyses		●	●	
Reduced representation techniques (GBS, RADseq)	●	●		●
Transcriptomes		●		●

Increased taxonomic/population coverage

Increased genome representation

A *geographic cline* can be defined as a consistent gradual change in gene frequency along a geographic axis, contrasting with phylogeographic discontinuities that are abrupt rather than progressive. the presence of clines in marine species suggests that gene flow is not always the strongest force influencing allele frequency changes; frequently, natural selection is powerful enough to overcome high migration rates. Clines are generated when selection at the edge of a geographical range acts in the opposite direction (primary differentiation) or by secondary migration between genetically distinct populations (Endler, 1977).

If migration occurs exclusively between neighbouring populations and distant populations are connected via intermediate '*stepping stones*', genetic differences between populations increase with increasing geographic distance. This pattern is also called isolation-by-distance (Wright, 1943). In stepping stone gene flow, estimates of pairwise gene flow are high for close populations but low for distant populations.

In some species with pelagic larvae, adult populations show low levels of genetic subdivision. However, repeated sampling of recruits from the same locations over time may show that different cohorts are genetically different. This pattern is known as *chaotic genetic patchiness*.

In the *broad-scale homogeneity populations*, also known as panmictic populations, species with planktotrophic larvae exhibit high genetic similarity across wide geographical scales, apparently due to ongoing gene flow, with numerous closely related alleles shared by distant populations.

Understanding the genetic characteristics of different populations can provide insights into a species's evolutionary history and population dynamics.

2.6. Molecular markers

Molecular markers are specific DNA or RNA sequences that can be used to identify and track genetic variation within and among populations. In population genetics, molecular markers are often used to study the distribution and frequency of genetic variation within and among populations and the processes that shape these patterns. There are several different types of molecular markers that can be used in population genetics studies. Both the mitochondrial and nuclear genomes have been intensively used in phylogeography. The different types of molecular markers generate data appropriate to address different questions or topics (Table 3). Mitochondrial genes or gene fragments more commonly used are the Cytochrome Oxidase I (COI), the Cytochrome b (Cytb), and the non-coding region D-loop. More recently, with the advent of high-throughput sequencing, it is possible to obtain data from thousands of single nucleotide polymorphisms (SNPs) interspersed in mitochondrial and nuclear genomes. One common application of molecular markers in population genetics is using genetic data to infer a species's evolutionary history and population dynamics. For example, molecular markers can be used to identify gene flow patterns between populations, estimate populations' size and structure over time, and identify the presence of genetic

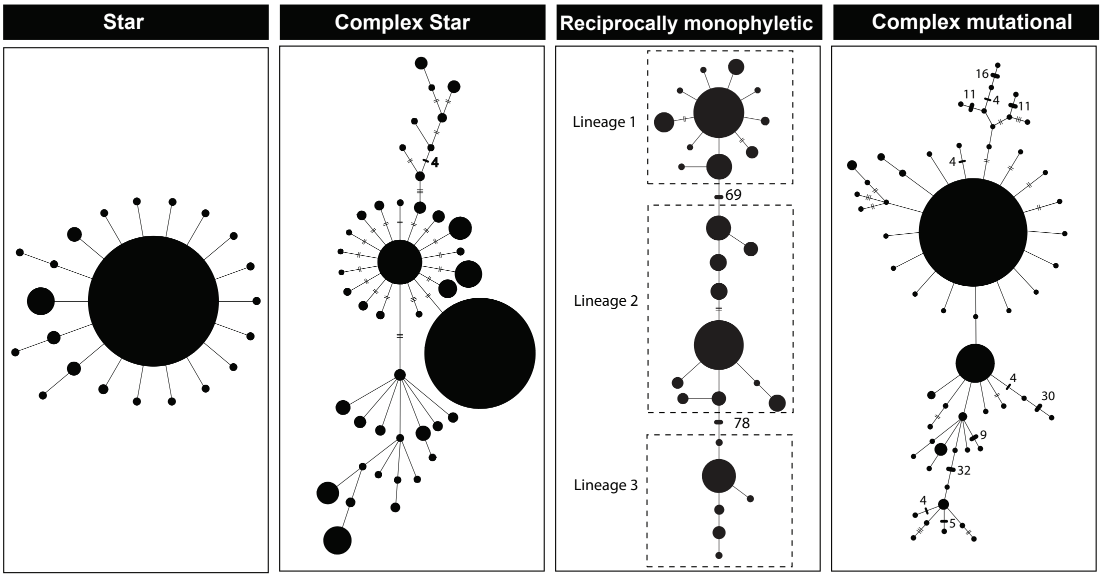


Figure 12. Each circle of the haplotype network represents a unique haplotype and the sizes of the circles are proportional to the haplotype frequencies Each line represents one mutation step and two or more steps are indicated by bars or numbers. Networks modified from Jenkins et al. (2018).

bottlenecks[1] or selective sweeps[2]. Molecular markers can also be used to study the genetic basis of ecologically important traits, such as resistance to disease or environmental stressors, or to identify the genetic basis of evolutionary adaptation to different environments. Overall, the use of molecular markers in population genetics has greatly increased our understanding of the processes that shape genetic variation within and among populations and has provided important insights into the evolutionary history and population dynamics of many species.

MtDNA haplotype networks

Because the case studies selected are based on data from the mitochondrial genome, it is appropriate to do a short introduction highlighting the most common concepts used. The mitochondrial genome is a well-known circular, small and haploid genome, which lends itself to phylogeographic approaches. Being a relatively small molecule (ca 16.000 base pairs) means that DNA primers (short sequences of nucleotides that are used to initiate the synthesis of a new strand of DNA of PCR) for specific regions of the mtDNA were easy to design, and many amplify diverse species (universal primers). By studying the variation in the mitochondrial genome of a species, scientists can infer past population movements, genetic isolation, and other evolutionary processes that have shaped the distribution of genetic variation. Because mtDNA is haplotypic, its genetic variants are called haplotypes, and the relationships between different haplotypes can be graphically represented by haplotype networks.

Haplotype networks are constructed using a variety of algorithms, but the most common approach is to use a method called statistical parsimony. This method starts with a set of observed haplotypes and uses a series of genetic mutations (such as single nucleotide polymorphisms) to reconstruct the most parsimonious evolutionary history of the haplotypes. The result is a network of haplotypes that are connected by edges representing genetic mutations. The network can be represented as a graph, where the haplotypes are represented as nodes (circles), and the edges represent the mutational steps between them. The network can infer relationships between haplotypes, such as common ancestry or recent gene flow, and estimate the relative age of different haplotype clusters. The nodes representing haplotypes can be displayed as pie charts, in which colours represent the geographical origin of the individuals sharing a haplotype.

The haplotype networks can be typified into four different groups (Figure 12):

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[1] A genetic bottleneck is a dramatic reduction in the size of a population that results in a decrease in genetic diversity. This can occur due to a variety of factors, such as natural disasters, habitat destruction, overhunting, or the introduction of a new species or pathogen.

[2] A selective sweep is a process that occurs when a particular genetic variant (allele) becomes fixed (present in all individuals) in a population due to strong natural selection.

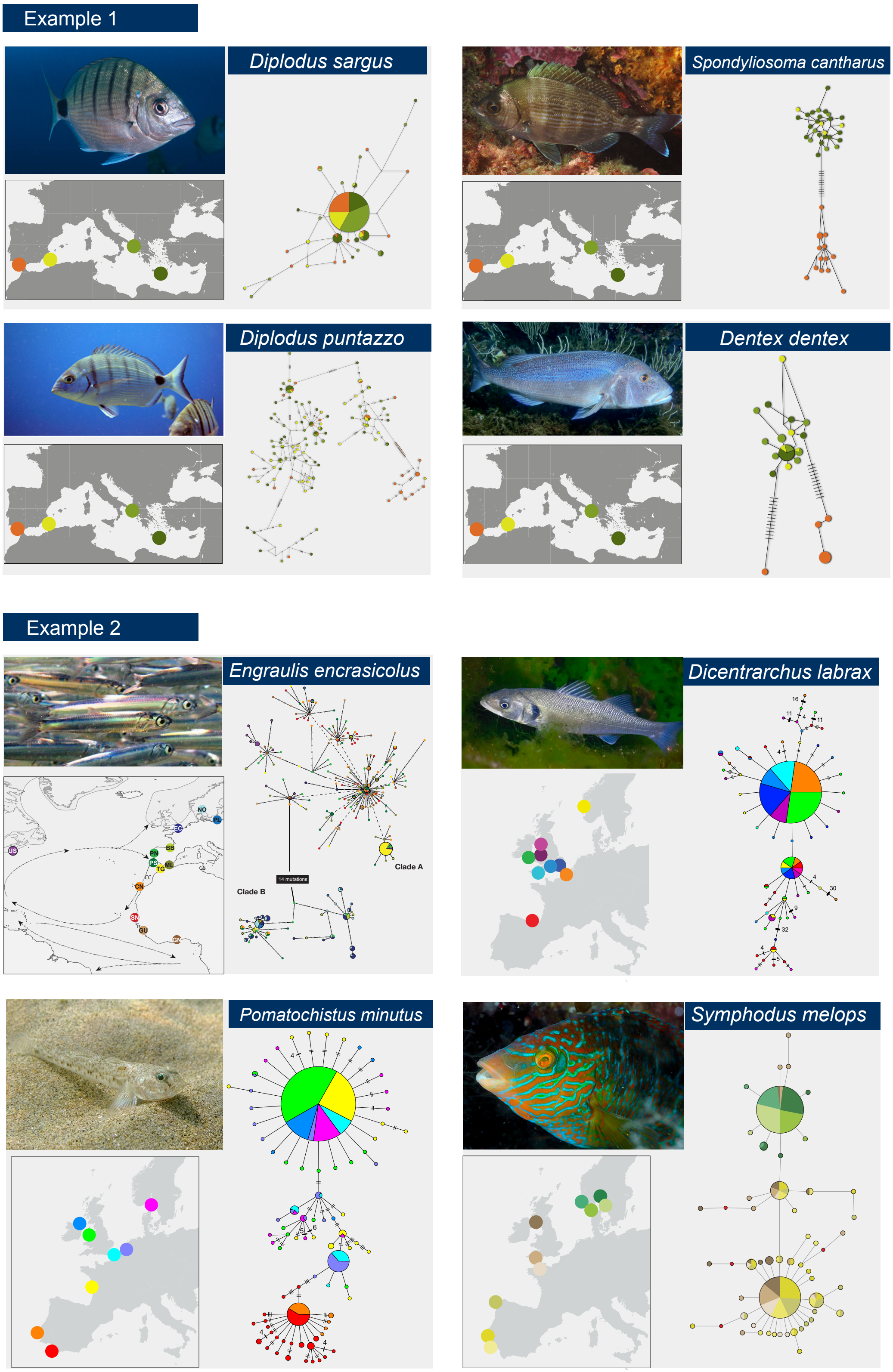

Figure 13. Example 1: Species of the Sparidae family displaying different patterns of differentiation across the Atlantic-Mediterranean. Example 2: Species displaying different levels of latitudinal genetic diversity promoted by the influence of the LGM.
Colors in haplotype networks represent geographical location of the individuals.Each circle of the haplotype network represents a unique haplotype and the sizes of the circles are proportional to the haplotype frequencies for each network but are not comparable across studies. Colours inside the circles correspond to sites which have individuals represented in that particular haplotype. Each line represents one mutation step and two or more steps are indicated by bars or numbers.

(1) A ‘star’ network, in which a single, widespread haplotype is typically positioned at the centre of the network and is thought to be the ancestral haplotype. Additional haplotypes are linked to this dominant haplotype by a single (or a few) mutational step(s), suggesting these haplotypes are the product of recent mutation events. Sometimes, the dominant haplotype has far fewer connections than a low-frequency haplotype in the network, making it difficult to distinguish the centre of the network with confidence;

(2) A ‘complex star’ network, in which there are multiple high-frequency haplotypes and connections;

(3) A ‘reciprocally monophyletic’ network, in which more than one lineage is apparent, and each lineage is linked by a long branch associated with numerous mutation;

(4) A ‘complex mutational’ network, in which some branches were separated by a very large number of mutations, while other branches had contrarily one or two mutations. In many cases, a dominant haplotype was present and was presumed to be the ancestral form.

3. Case-studies

To present the phylogeography of the Northeastern Atlantic Ocean and de Mediterranean Sea, I will expose studies that explored the effect of the last glaciation on the marine fish fauna as an example of the integration of molecular data, habitat data and species distribution data. I will dwell on the intraspecific genetic diversity representing each species' evolutionary and adaptive potential in changing environments. Phylogeography addresses, among others, the following over-arching questions: (1) How have historical events shaped the species distribution and the genetic diversity observed patterns? (2) Do areas of glacial refugia coincide with areas harbouring higher genetic intraspecific diversity?
During the LGM, a period of global cooling and glaciation, sea levels were much lower than they are today, and large areas of the continental shelf were exposed. This significantly impacted marine species' genetic diversity, as populations were separated and isolated in different areas. The Last Glacial Maximum (LGM) significantly impacted the genetic diversity of marine species, leading to changes in the latitudinal distribution of genetic diversity for many species. Because the LGM occurred approximately 21,000 years ago, its influence is still clearly imprinted in the genetic makeup of present-day species.

To address these questions and illustrate the large body of work on fish phylogeography of the Northeastern Atlantic Ocean and the Mediterranean Sea, I have chosen case studies that represent the clear or equivocal influence of the Atlantic-Mediterranean divide and the different levels of latitudinal genetic diversity.

In example 1 (Figure 13), four species of the family Sparidae display contrasting patterns of genetic diversity and genetic differentiation across the Atlantic-Mediterranean transition. The difference in the four species' genetic differentiation patterns is clear. While the *Diplodus diplodus* and *D. puntazzo* species display no Atlantic-Mediterranean differentiation, with haplotypes (mitochondrial genetic variants) from the Atlantic and Mediterranean grouped together, the *Spondyliosoma cantharus* and *Dentex dentex* show a clear separation between Mediterranean and Atlantic haplotypes. What may explain these different results of species of the same family? The Sparidae larvae can generally be divided into two groups: “small Sparidae” (i.e., 9–11 mm in standard length at settlement) and “large Sparidae” (i.e., 14–16 mm in standard length at settlement). Small Sparidae larvae are slenderer and apparently less developed at settlement than those in the large Sparidae group. Small Sparidae also swims slower than large Sparidae (11.1 vs 19.2 cm s−1; Faillettaz et al., 2017), and their pelagic larval duration is shorter (14–18 d on average vs 30–38 d; Raventós and Macpherson, 2001; Macpherson and Raventos, 2006). Both *Diplodus* species belong to the small Sparidae group, while *Spondyliosoma cantharus* and *Dentex dentex* belong to the large group.

In example 2, the remarkable mobility of European anchovies (*Engraulis encrasicolus*) has enabled them to pursue their preferred thermal physiological conditions amidst the severe climate changes of the Last Glacial Maximum, thus evading significant population declines and genetic bottlenecks (Silva et al., 2014); the seabass (*Dicentrarchus labrax*) (Coscia & Mariani, 2011),sand goby (*Pomatochistus minutus*) (Larmuseau et al., 2009) and the corkwing wrasse (*Symphodus melops*) (Robalo et al., 2012) all display higher haplotype diversity in the southern locations, and a relatively genetically impoverished in the northern locations an evident indication of the imprint of the LGM on the genetic mackup of these species.

Overall, these studies highlight the significant impact that the LGM had on the distribution and genetic diversity of marine Atlantic species. These changes have had long-lasting effects on the genetic structure of these populations and have important implications for their conservation and management.

4. Are generalisations possible?

We showed that generalisations regarding different species' genetic differentiation patterns are possible. However, much baseline biological data and general knowledge on marine or non-fish species are missing. For instance, the lack of general knowledge on pelagic larvae duration and near-coastal oceanographic movements hinders our ability to identify the main drivers of dispersal or retention. Although the existence of soft barriers such as gyres or other oceanographic fronts may promote the retention of organisms on either side of the barrier, the fact is that those barriers are porous and non-universal, and different species will react differently to those gene flow limitations.

Latitudinal genetic differentiation patterns in marine species during the Last Glacial Maximum (LGM) varied depending on the specific species and their distribution ranges. However, some generalisations can be made. In general, species that were distributed across a wide latitudinal range exhibited lower levels of genetic differentiation than species with more restricted distributions. This is because species with broad distributions were able to track changes in environmental conditions during the LGM by shifting their ranges, whereas species with more restricted ranges were more likely to become isolated from each other and experience genetic divergence. Additionally, some studies have suggested that the latitudinal gradient in species richness and diversity may have been steeper during the LGM than it is today. This is thought to be due to the contraction of species' ranges towards the equator, leading to increased competition and the exclusion of some species from higher latitudes. Overall, the latitudinal genetic differentiation patterns observed in marine species during the LGM were influenced by a complex interplay of historical and contemporary factors, including ocean currents, dispersal ability, habitat connectivity, and species-specific ecological requirements. Understanding these patterns can help us better understand marine species' evolutionary history and how they may respond to future environmental change.

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