INVITED REVIEW
Pillars of Hercules: is the Atlantic–Mediterranean transition a phylogeographical break?

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Abstract
The geological history of the Mediterranean Sea, its hydrography and connection with the Atlantic Ocean have been well documented. Despite a wealth of historical and oceanographic data, the Atlantic–Mediterranean transition remains controversial at the biological level as there are discordant results regarding the biogeographical separation between the Atlantic and Mediterranean biota. The opening of the Strait of Gibraltar at the end of the Messinian Salinity Crisis (some 5.33 million years ago), removed the land barrier that impeded the marine biota allowing it to disperse freely into the Mediterranean Sea. However, present day genetic patterns suggest a limitation to gene flow for some marine species, preventing population admixture. In the last few years, a large number of studies have challenged the hypothesis of the Strait of Gibraltar representing a phylogeographical break. A review of more than 70 papers reveals no obvious relationship between either dispersal ability or life history, and observed patterns of partial or complete genetic isolation between Atlantic and Mediterranean populations. We re-analysed a selection of this large body of data (20 studies in total) in order to provide a homogeneous and coherent view on the generality of the phylogeographical patterns and the presence of a phylogeographical barrier. This offered the opportunity to summarize the state of the art on this matter and reach some general conclusions on the evolutionary history across the Atlantic–Mediterranean range. Geographically, some species in the transition zone showed step changes of allele frequencies associated with the Almeria-Oran Front rather than with the Strait of Gibraltar itself. A major part of the data describe evolutionary events well within the time frame of the Quaternary age as very few taxa pre-date closure of the Tethys Sea. Results point to a combined signature of vicariance, palaeoclimate fluctuation and life-history traits on the Atlantic–Mediterranean phylogeographical patterns. Principal component analysis failed to show any particular association between biological traits and genetic variables. It would argue that organismal determinism may play a far less significant role than marine biogeographers have generally believed.

Keywords: Almeria-Oran front, Atlantic Ocean, evolution, Mediterranean Sea, phylogeography, population genetics, Strait of Gibraltar

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Phylogeography in the marine realm
Origin and maintenance of genetic diversity is a central issue in evolutionary biology. Understanding these processes in the ocean realm is particularly difficult because barriers to gene flow are far less obvious in marine compared to continental species. Vicariance is usually invoked as the most likely model of speciation promoting genetic discontinuities across geographical ranges (Cunningham & Collins 1998). However, historical environmental factors related...
to habitat, currents and glaciations (Roy et al. 1996; Wares 2002) combined with species-specific traits play a pivotal role in shaping the pattern of inter- and intraspecific differentiation. Their relative contribution remains hard to disentangle. These ancestral interactions combined with present-day environmental patterns are the focus of marine phylogeographical investigations.

Taxa with congruent geographical patterns of intra-specific structure have provided evidence as well as documented cases of phylogeographical breaks in the marine environment. Both ancestral and contemporary discontinuities in the marine environment are, for example, reflected in the biogeographical realm (Longhurst 1998). Well known cases are ancient breaks between the Southern Ocean and the surrounding oceanic waters associated with the formation of the Antarctic Polar Front some 20–30 million years ago (Ma) (Bargelloni et al. 2000), as well as the Equatorial Pacific and Atlantic Ocean at the Isthmus of Panama some 3.1–3.5 Ma (Avise 2000). Breaks of a more recent date are located between the Baltic Sea and the North Sea some 7500 years ago (Olsen et al. 2004), the northern and southern Atlantic Shelf (Cape Hatteras) where the Labrador Current and Gulf Stream have met on and off during the Quaternary (Weinberg et al. 2003). Sharp genetic breaks were also documented in the Indo-Pacific region north and south of the Flores and Java Seas (Barber et al. 2000), and at Cape Canaveral in the Florida Keys (reviewed in Avise 1992). Many of these cases turned out to be influenced by the large climatic fluctuations during the Quaternary. These fluctuations left a detectable trace in the genomic architecture of the contemporary terrestrial (Hewitt 2000; Soltis et al. 2006) and freshwater fauna (e.g. Bernatchez 2001), and less so in the oceans because of the ability of offshore organisms to move latitudinally (Cunningham & Collins 1998).

Biogeographical analysis focused on the Gulf of Mexico–Florida range revealed that several species of invertebrates and vertebrates have concordant genetic separation with reciprocal monophyly, indicative of a deep historical partition. However, not all taxa showed the same population subdivision. In fact, some species occupying the same area revealed very shallow to no phylogeographical pattern. Interestingly, neither the present-day distribution pattern (continuous or discontinuous) across the geographical boundary of Southern Florida, nor the dispersal abilities were good indicators of the genetic structure displayed by each taxon (Cunningham & Collins 1998). This suggests that shared biological features between species do not necessarily imply similar population structure. The lack of life-history characteristics associated with patterns of population subdivision at Cape Canaveral is consistent with a hypothesis that the vicariant events affected many species without regard to their life history. The reciprocal monophyly between populations of the southeast Atlantic coast and the Gulf of Mexico reported for a number of unrelated species can be associated with the same historical process (Avise 2000). However, as this is not always the case, the question arises why in some species such a historical event has left clearly detectable and concordant signs, whereas in other species it has not? Why did vicariance, separating populations across a given boundary, not equally affect all species over the same geographical range? Two possible explanations were given; the simplest and more intuitive is that taxa with higher dispersal ability are expected to have less pronounced population structure but, this does not hold true for the species studied north and south of Cape Canaveral (see above) since almost all of them have planktonic larvae that potentially contribute to high dispersal (Cunningham & Collins 1998). For instance, fish with equally high dispersal ability show either cases of reciprocal monophyly (e.g. black sea bass and toadfish) or shallow population structure (e.g. sturgeon and menhaden) with evidence of a recent genetic connection between the Atlantic Ocean and the Gulf of Mexico (Avise 1992). Extinction followed by recolonization is an alternative explanation to justify the absence of genetic subdivision among populations. Even assuming that cladogenetic processes have determined genetic discontinuity across a geographical boundary, extinction of one of the two lineages, followed by recolonization by members of the other lineage results in a genetic homogenization over the entire distribution range of the species (Wares & Cunningham 2001). The genetic signature of the recent extinction/recolonization is detectable as (i) reduced genetic diversity in the colonized area compared to the source population, and (ii) similar allele phylogenies on both sides of the geographical boundary.

Biogeographical origins of the Mediterranean biota

The term ‘biodiversity’ usually refers to species richness but it encompasses several interrelated aspects of biological diversity ranging from population to ecological communities. Although in this review we will develop those aspects of biodiversity related to population genetics, thus concentrating on intraspecific diversity, it is worth providing an overview of the species richness and diversity as well as a historical context of the Mediterranean Sea.

More than 8500 species of macroscopic organisms have been reported in this semiclosed sea (Longhurst 1998; Bianchi & Morri 2000), representing between 4% and 18% of the world’s marine biodiversity. Interestingly, this value of species diversity seems rather high for a basin representing only 0.82% of the surface area and 0.32% of the volume of the world’s oceans. In addition, more than one-quarter of the species are endemic to the Mediterranean (Tortonese 1985). This high biodiversity could be attributed to the historical interest in the Mediterranean Sea, but it may also have its roots in the troubled geological history of the Mediterranean region (Fig. 1). The Mediterranean Sea...
ranged, in the last few million years, from almost complete desiccation during the ‘Messinian salinity crisis’ (MSC) (up to 5.33 Ma) to cycles of cold glacial periods followed by warm interglacial periods during the Quaternary (Box 1). The present-day biota are largely the result of colonization, mostly from the Atlantic Ocean (Almada et al. 2001; Domingues et al. 2005) and to a minor extent from the Red Sea. In fact, after an isolation and desiccation period that lasted about 0.5 million years, re-flooding of the Mediterranean basin was possible because of the inflow of Atlantic waters through the newly opened Straits of Gibraltar. The MSC drove the pre-existing Indo-Pacific biota to extinction, with the few exceptions of taxa of Miocene origin that have survived in shallow-water refuges, for example killifishes (Hrbek & Meyer 2003).

This geographical history suggests that species communities contemporarily inhabiting the Mediterranean waters can be divided into the following biogeographical categories: (i) temperate Atlantic-Mediterranean species; (ii) cosmopolitan/panoceanic species; (iii) endemic species, including palaeo-endemic (Miocene) and neo-endemic (Pliocene) species; (iv) subtropical Atlantic species (inter-glacial remnants); (v) boreal Atlantic species (glacial remnants); (vi) Red Sea invasive (Lessepsian) species entering through the Suez Canal; and (vii) eastern Atlantic invasive species. Bianchi & Morri (2000) identified 10 biogeographical regions in the Mediterranean according to the relative abundance of each of the aforementioned categories. Transition from one biogeographical zone to another results from a combination of geological (orogenesis and hydrogeology), physical (present-day hydrography, coastal and seabed profile) and biological factors (species biology and evolutionary history), all contributing to shape the intra- and interspecific diversity.

Population structure across the Atlantic–Mediterranean transition

The information reported above, although pertaining to interspecific diversity, is useful to set a historical and biogeographical context for the Mediterranean Sea and adjacent regions. However, the goal of this work is not to investigate differences between species but rather to concentrate on the geographical partitioning of genetic
Box 1 Mediterranean palaeoceanography

Four features dominate the palaeoceanography of the Mediterranean Sea: (i) it is enclosed geographically with the presence of sills separating individual basins; (ii) its connection to the Atlantic Ocean — throughout history the main source of biota for colonization; (iii) the climate regime where temperature determines the natural range of living organisms, and where the temperature and evaporation balance determine seawater density and hence thermohaline circulation; and (iv) the sea level, which determines hydraulic connectivity between basins.

Enclosed geography

The Mediterranean Sea is a fully enclosed sea, except for a 12.9-km wide and 286-m deep connection with the Atlantic Ocean between Cape Trafalgar and Cape Spartel (Straits of Gibraltar) (Figs 1 and 2). The Mediterranean Sea has a depth of approximately 3400 m in the western basin and 4200 m in the eastern basin; therefore, it can be named a proper ocean basin. It is divided into two units by a shallow sill (350 m deep) in the Strait of Sicily, namely the Eastern and the Western Mediterranean. The Bosporus–Dardanelles sill (40–70 m) separates the Black Sea from the Mediterranean Sea. The Pelagosa sill (160 m) separates the central Adriatic Sea from the Eastern Mediterranean Sea. The man-made Suez Canal (opened in 1869) in the southeastern Mediterranean Sea has led to a steady influx of saline water (and biota) from the subtropical Red Sea.

Connection to the Atlantic Ocean

Apart from the Suez Canal, the only source of oceanic water to the Mediterranean Sea originates from the North Atlantic Ocean, entering through the Straits of Gibraltar. The Mediterranean Sea became stably separated from the Atlantic Ocean during the Messinian salinity crisis (MSC), which occurred in the late Miocene (5.59–5.33 Ma) (Hsü et al. 1973; Krijgsman et al. 1999). The sea level of the Mediterranean Sea dropped dramatically. Once the obstruction to this gateway was removed because of tectonic uplifting, faulting and sea level changes, the Mediterranean Sea was flooded catastrophically and synchronously with oceanic water, one of the most dramatic events of the Cenozoic (Fig. 1a–c). Recurrent, short periods of separation between the Atlantic and Mediterranean waters occurred during the Quaternary in correspondence to cyclic ice ages and the associated sea level changes (Fig. 1d).

Climate regime

The relatively small and marginal basin of the Mediterranean Sea has tracked global climate oscillations faithfully. The Lago-Mare facies during the MSC represented evidence of extensive desiccation and the formation of hypersaline and freshwater sub-basins (Krijgsman et al. 1999). Although large areas of low salinity were present in the proximity of river outflows, few coastal marine taxa survived this period (Penzo et al. 1998; Hrbek & Meyer 2003; Huyse et al. 2004). Termination of the MSC coincided with the catastrophic flooding of the Mediterranean Sea. Following the restoration of the marine ecosystem, the Late-Pliocene epoch saw several climate-induced increases in marine production associated with processional minima and coincidental regional warming (Passier et al. 1999; Haywood et al. 2000; Meyers & Arnaboldi 2005). About 3 Ma, the Mediterranean climate was warmer and wetter than today (Haywood et al. 2000). During the Quaternary, the Mediterranean Sea continues to register and amplify the smallest climatic variations occurring at mid-latitude regions (Sbaffi et al. 2001). Hence, the glacial–interglacial cycling so typical during the Pleistocene is well reflected in the palaeontological record. Relatively warm periods (interglacials) have been dated at ~630 000, ~330 000, ~200 000, ~130 000 (the last interglacial) and 11.500 years ago (the current interglacial, named Holocene). Glacial stadia, including the last glacial maximum (LGM) — 24 000–20 700 years ago (Lambeck et al. 2002) have led to colder (but ice-free) conditions. Local analysis of dated deposits by means of proxies of sea surface temperature, point to a close tracking of the climate by the biota (Zonneveld 1996; Paterne et al. 1999; Sbaffi et al. 2001; Rohling et al. 2002).

During the Pleistocene period, circulation in the Mediterranean Sea became so restricted at times that the deeper waters became anoxic as in the Black Sea today. Primary productivity of the basin increased and a series of organic rich sediments, called sapropels, were deposited (e.g. at 10 500 and 6100 years ago) (Krom et al. 2001; Rohling et al. 2002). The climate around the basin was less arid; there was increased rainfall in central and eastern Africa and a higher river flow in the Nile. It is not clear whether the circulation remained anti-estuarine or became estuarine. Such climate changes often translated to an impoverishment in taxa as there was no latitudinal range to track the isothermals. Hence, extinction and recolonization have played a major role in the Mediterranean Sea (Cunningham & Collins 1998).

Currently a Mediterranean climate dominates the region, that is wet cool winters and dry warm summers, with a total precipitation of 420 mm a−1. River discharge
into the Mediterranean Sea is dominated by rivers from the northern temperate zone, such as the Ebro, Rhône, Po and Seyhan, and especially from the East and Central European rivers flowing into the Black Sea (Danube, Dnjestr, Dniepr and Don). Runoff from the arid southern (sub)tropical zone is very reduced (e.g. Nile), such that there is a 1:5 shortfall in precipitation and runoff over evaporation.

Sea level

Sea level changes are related to tectonics, solar modulation of climate (and ice sheets), geoid and deformation of ocean basins (Lambeck et al. 2002). They have globally and locally had a major effect on the oceans and especially the continental shelves. After the dramatic events of the MSC, sea levels have changed regularly throughout the Pliocene and Pleistocene. The lowest sea levels were observed 140 000 years ago (~130 m) and 30 000 years ago (~120 m) (Fig. 1d). The Mediterranean Sea with its sill determined topography has directly felt the impact of changing sea levels through changing flow regimes between the Black Sea, the Eastern Mediterranean, the Western Mediterranean and the Atlantic Ocean. The Black Sea was separated on and off from the Mediterranean Sea through exposure of the shallow sills at the Straits of Dardanelles and Bosporus (Aksu et al. 2002).

Fig. 2  Schematic representation of the main currents characterizing water circulation in the Mediterranean and Black Sea.

diversity within species across Atlantic–Mediterranean transition zones. For this purpose, we consider three major geographical provinces: the eastern Atlantic, the western Mediterranean and the eastern Mediterranean, that largely follow the bathygraphy (Fig. 2). Intraspecific phylogenetic breaks and/or genetic transitions will be considered in association with two main geographical boundaries separating the aforementioned provinces: the Straits of Gibraltar (dividing the Atlantic Ocean and western Mediterranean Sea) and the Strait of Sicily (separating the eastern and western Mediterranean). The geological history of the Mediterranean Sea has been well documented (Fig. 1) as has its present-day hydrography (Fig. 2) within the Mediterranean and between the Mediterranean and the Atlantic Ocean (see Box 1 and Box 2). Despite the wealth of geological and oceanographic data, the Atlantic–
Mediterranean transition at the biological level is controversial, as there are discordant results regarding the biogeographical separation between the Atlantic and Mediterranean biota. The opening of the Straits of Gibraltar at the end of the MSC allowed free dispersal of the marine biota between the Atlantic Ocean and Mediterranean Sea. Since then, several geological events influenced the history of the Mediterranean region, most importantly recurrent glaciations during the Pleistocene.

In the past few years, the hypothesis that the Straits of Gibraltar acts as a barrier to gene flow has been tested in many organisms including plants and marine mammals. However, the rapidly growing literature on the Atlantic–Mediterranean divide is largely based on a ‘single marker–single species’ scheme. Differences in the biology of species combined with differences in the data analysis make comparison between studies complex. To compensate for this discrepancy, we performed a re-analysis of 20 selected species (3 invertebrates and 17 vertebrates) over the target geographical range (see Table 1 and ‘Data re-analysis’ section below). In particular, we consider case reports on those situations that fit three basic scenarios: (i) no or weak population structure; (ii) population structure but no sign of Mediterranean–Atlantic separation; (iii) population structure with significant differentiation between Mediterranean and Atlantic populations. In the latter case, further substructuring at a smaller geographical scale within each basin may also be observed.

Atlantic–Mediterranean differentiation

Previously, population differentiation across the Atlantic–Mediterranean transition was described in a number of marine species (Borsa et al. 1997), with extreme cases of reciprocal monophyly (see below and Table 1). Investigations were carried out both at the large and fine geographical scale providing phylogeographical scenarios of various degrees of detail. Population surveys over a wide geographical range spanning the northeast Atlantic Ocean, the Mediterranean sea (west and east) and the Black Seas were reported for two sea grass species (Zostera marina and Z. noltii, Coyer et al. 2004; Olsen et al. 2004), for the blue mussel (Mytilus galloprovincialis, Ladoukakis et al. 2002), for the chaetognath (Sagitta setosa, Peijnenburg et al. 2004, 2006) and for the anchovy (Engraulis encrasicholus, Magoulas et al. 2006). Mitochondrial DNA analysis showed a very similar pattern in all species with distinct gene pools associated with each of the three basins. The strongest genetic separation was observed in the two invertebrate species which showed complete lineage sorting between Atlantic vs. Mediterranean and Black Sea samples.
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<td>Pagrus pagrus</td>
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<td>160</td>
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<td>Bargelloni et al. 2003‡</td>
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<td>Paracentrotus lividus</td>
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<td>COX1</td>
<td>127</td>
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<td>Duran et al. 2004d</td>
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<td>168</td>
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<td>Rios et al. 2002†</td>
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<td>No</td>
<td>Borsa et al. 1997†</td>
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<td>Cytochrome b</td>
<td>272</td>
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<td>Stefani &amp; Thorley 2003‡</td>
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<td>Raja clavata</td>
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<td>Cytochrome b,</td>
<td>385</td>
<td>Yes; yes</td>
<td>No</td>
<td>Chevolot et al. 2006</td>
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Table 1 List of candidate papers considered for the present review and summary of the relevant information reported in each work.
Table 1  Continued

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<th>Species</th>
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<td>Sagitta setosa</td>
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<td>Yes (1.7)</td>
<td>Peijnenburg et al. 2004†</td>
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<td>Viñas et al. 2004*</td>
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<td>Atarhouch et al. 2006‡</td>
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<td>20</td>
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<td>263</td>
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<td>—</td>
<td>Zardoya et al. 2004¶§</td>
<td>Yes (18)</td>
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<td>D-loop</td>
<td>50</td>
<td>Yes; yes No</td>
<td>Nesbo et al. 2000¶</td>
<td>Yes (17)</td>
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<td>Microsatellites</td>
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<td>Perez-Losada et al. 2002†</td>
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<td>Fish</td>
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<td>259</td>
<td>Yes; weak —</td>
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<td>Alarcon et al. 2004‡</td>
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<td>Microsatellites</td>
<td>361</td>
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<td>De Innocentiis et al. 2004‡</td>
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<td>Yes; yes Yes (1.2–1.8)</td>
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<td>Garcia-Martinez et al. 1999†</td>
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<td>143</td>
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<td>Obst et al. 2005*</td>
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<td>101</td>
<td>No</td>
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<td>Costagliola et al. 2004*</td>
<td>No</td>
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<td>188</td>
<td>No</td>
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<td>Pujolar et al. 2003†</td>
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<td>179</td>
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<td>Ely et al. 2002¶</td>
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<td>783</td>
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<td>182</td>
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<td>—</td>
<td>Karaiskou et al. 2004¶</td>
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<td>140</td>
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<td>Karaiskou et al. 2004‡</td>
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<td>145</td>
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<td>Natoli et al. 2005**</td>
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<td>120</td>
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<td>Chow et al. 2000‡</td>
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<td>401</td>
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<td>—</td>
<td>Pujolar et al. 2002‡</td>
<td>No</td>
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<td>17</td>
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<td>Dalebout et al. 2005*</td>
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<td>Microsatellites</td>
<td>1706</td>
<td>Yes; no</td>
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<td>Coyer et al. 2004</td>
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<td>1756</td>
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<td>ITS-1, 2</td>
<td>383</td>
<td>Yes; no</td>
<td>—</td>
<td>Olsen et al. 2004†</td>
<td>No</td>
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</table>

*Data not used because of incomplete geographical sampling coverage, insufficient number of individuals or extremely low polymorphism; †data type other than mitochondrial DNA sequences [allozymes, single-strand conformation polymorphism (SSCP), restriction fragment length polymorphism (RFLP), microsatellites etc.]; ‡two subspecies with disjunct geographical distribution, therefore configuring a case of incomplete geographical sampling coverage; §samples from Nesbo et al. 2000 included; ¶datafile provided by authors; **datafile reconstructed from sequences in GenBank; ††samples from the Alboran Sea group with the Atlantic samples; N.A., information not available. In the last column, numbers in parenthesis refer to Fig. 4 and Tables S2 and S3.

Mediterranean and Black Sea populations appeared more closely related in both *M. galloprovincialis* and *S. setosa*, suggesting that the Black Sea populations originated from ancestral Mediterranean populations. In *S. setosa*, the separation between Atlantic and Mediterranean lineages was estimated to have occurred about 1.7 Ma, whereas divergence between Mediterranean Sea and Black Sea lineages was as recent as 400 000 years ago. These time estimates may be imprecise considering that they were based on a 2% mutation rate for the entire mtDNA. However, they are in agreement with similar observations reported for two copepod species *Calanus helgolandicus* and *C. euxinus*, inhabiting the Mediterranean Sea and the Black Sea, respectively, which diverged during the Pleistocene (Papadopoulos et al. 2005). Three well-resolved groups were also identified in the two species of *Zostera*, matching three geographical areas: Northern Europe, Mauritania and the Black/Azov Sea. The Mediterranean samples were separated from all the others and placed intermediate between the northern Atlantic and the Black/Azov Sea populations accounting for a general scheme of ‘isolation by distance’. Anchovy populations appeared more structured with some puzzling haplotype distribution. However, four main geographical groups were clearly identified: Atlantic, central Mediterranean, Aegean Sea, and Black Sea. Interesting is the evidence produced to support the affinity of the alboran sea samples to the atlantic samples. Large-scale population surveys were also carried out in the
European hake, Merluccius merluccius over the entire distribution range of the species, which extends form the northeast Atlantic to eastern Mediterranean (Lundy et al. 1999; Cimmaruta et al. 2005). This commercially valuable species also showed clear separation between Atlantic and Mediterranean stocks detectable by both microsatellites and allozymes. Interestingly, allozyme pattern showed a strong correlation with salinity and temperature suggesting a role for these environmental factors in maintaining the genetic differentiation among the two population groups through selective processes (Cimmaruta et al. 2005). Genetic discontinuity between Atlantic and Mediterranean hake populations was observed to correspond with the Almeria-Oran front.

At the fine geographical scale, the Atlantic–Mediterranean transition area was investigated in the cuttlefish (Sepia officinalis, Perez-Losada et al. 2002), the blue mussel (M. galloprovincialis, Quesada et al. 1995), the sea urchin (Paracentrotus lividus, Duran et al. 2004b) and the European sea bass (Dicentrarchus labrax, Lemaire et al. 2005). In all cases, sampling was aimed at providing a detailed population analysis of the region. With the exception of the sea urchin, which showed a slight (although significant) differentiation between basins, the three other species revealed significant clinal changes in the transition from Atlantic to Mediterranean populations. More importantly, all species showed an abrupt ‘step’ change in allele frequencies associated with the Almeria-Oran Front (AOF) (Box 2). Such genetic discontinuity was clearly detectable irrespective of the molecular marker used (mtDNA for M. galloprovincialis and D. labrax, microsatellites for S. officinalis). A number of other species showed significant differences between Atlantic and Mediterranean populations as summarized in Table 1. In the next section, we will focus on cases where closely related species show opposite situations.

Contrasting results in closely related species

A useful approach for identifying common phylogeographical signals may come from the comparison between closely related species with a comparable biology. One may assume that with biological traits (i.e. life history and dispersal ability) being similar as much as possible, it should be more straightforward to identify a shared history of habitat fragmentation. From this perspective, the most extensively investigated group is the family Sparidae, a highly diversified group of demersal perciform fish (Bargelloni et al. 2003; Alarcon et al. 2004; De Innocentiis et al. 2004; Bargelloni et al. 2005). Bargelloni et al. (2003) surveyed population structure in Dentex dentex, Lithognathus mormyrus, Pagellus bogaraveo, Pogrus pagrus and Spondylisoma canthus with the same mitochondrial fragment (D-loop) and the same set of nuclear (allozyme) markers. The sampling scheme largely overlapped in order to maximize comparability. The results showed stark discordance among species. Three of the five species (De. dentex, L. mormyrus and Sp. canthus) revealed a sharp Atlantic–Mediterranean separation, whereas the other two (P. bogaraveo and Pa. pagrus) showed very little or no population structure and no signs of Atlantic–Mediterranean division. Concordance of De. dentex, L. mormyrus and Sp. canthus in reciprocal monophyly between Atlantic and Mediterranean populations suggested that the same historical biogeographical factors might have influenced intraspecific patterns of genetic differentiation. Further support for this view was given by the estimates of divergence time between Atlantic and Mediterranean clades. In fact, both De. dentex and L. mormyrus showed a gene flow extremely limited or absent between the two basins over the past 1.2–1.8 Myr. Such a temporal (early Pleistocene) and spatial (Strait of Gibraltar-AOF) placement of a phylogeographical boundary concurs with geological data (Nilsson 1982). The data confirm that climate fluctuations during the entire Quaternary period have produced episodes of habitat fragmentation between the Atlantic and the Mediterranean (Box 1). On a shorter timescale, some hydrological features such as the AOF might have reduced gene flow between both sides of the Strait. An analogous incongruent phylogeographical pattern was described for two sparids belonging to the same genus, Diplodus puntazzo and Dip. sargus. They share a similar ecological behaviour and have no or little differences in biological traits (Bargelloni et al. 2005). However, the pronounced population structure of Dip. puntazzo contrasts sharply with the little intraspecific differentiation in Dip. sargus. The opposite situation for the two congeneric species was interpreted as a consequence of differences in the population dynamics, such as fluctuation in the effective population size due to bottlenecks and expansions. Sparids are not the only example of differences in population structure between closely related species. Discordance was recently reported for two species of anglerfish (Lophius budgessa and Lo. pescatorius) (Charrier et al. 2006) and two species of mackerel (Scomber japonicus and Sc. scombrus) (Zardoya et al. 2004). Also, the comparative phylogeography of two large pelagic fish, the Atlantic bluefin tuna (Thunnus thynnus) and the swordfish (Xiphias gladius), reveals contrasting results (Alvarado-Bremer et al. 2005). Both species were characterized by a complex haplotype structure with two distinct clades within each species. However, no obvious geographical partition of D-loop haplotypes was observed for the bluefin tuna samples. Deep clades in the swordfish reflect a history of vicariant separation between the Atlantic Ocean and Mediterranean Sea. The proposed explanation for the observed difference between the two species relates to differences in their demographic history. Temperature fluctuation that characterized the Mediterranean Sea during the Pleistocene could have prevented bluefin tuna from reproducing and occupying the areas that this
species only recently re-colonized. On the contrary, swordfish adapted to Mediterranean Pleistocene conditions, possibly occupying a refuge area in the eastern Mediterranean where it remained isolated from the Atlantic population for the last 0.7 million years.

The literature on invertebrates largely consists of ‘single marker–single species’ studies. The only case that allows comparison between closely related species are two lobster species, the Norway lobster (Nephrops norvegicus) and the European lobster (Homarus gammarus). They belong to the same family and exhibit a very similar biology. They were investigated in two papers, both using a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) approach, applied to a mtDNA fragment (Stamatis et al. 2004; Triantafyllidis et al. 2005). Population structure of the Norway lobster was analysed across a wide geographical range encompassing the North Sea, Irish Sea, Portuguese coast and Aegean Sea (Stamatis et al. 2004). No signs were detected of Atlantic–Mediterranean differentiation or isolation by distance. The absence of a clear pattern of geographical structure for this burrowing crustacean with pelagic larvae might be only partly justified by gene flow over distances as far apart as the Aegean Sea and North Sea. In fact, a more likely explanation is a recent expansion. This possibility is supported by the relatively shallow haplotype network and by the mismatch analysis, which calculates the period of expansion to be as old as 280 000–440 000 years. In contrast, pronounced geographical structuring was reported for the European lobster (Triantafyllidis et al. 2005) whose range of distribution considerably overlaps with N. norvegicus. At least three well-differentiated groups were identified in H. gammarus, with the Mediterranean being well differentiated from all Atlantic samples. This species also showed significant substructuring within the Mediterranean. Notably, the Adriatic population appeared genetically divergent from all the others as reported for other invertebrate populations inhabiting this semiclosed sea suggests that the Adriatic Sea might represent a region of further phylo-geographical discontinuity deserving an in-depth analysis.

Data re-analysis

We prepared a meta-analysis of the most complete and accessible data sets to provide an integrated picture of Atlantic–Mediterranean phylogeography. Representative, balanced data were selected both in terms of species, sampling coverage and molecular markers that would enable the search for common historical patterns. We have thus selected all species for which mitochondrial DNA sequences were available (mtDNA offers the opportunity to compare the evolutionary history, giving a fair degree of population-level resolution), with samples from the northeast Atlantic Ocean and from the Mediterranean Sea, and with at least 10 individuals in each main basin (Atlantic and Mediterranean). These criteria proved to be quite stringent; from the initial 68 candidate data sets (Table 1), 20 were selected for the meta-analysis. They were kindly provided by the authors of the original publications (see Acknowledgements). A standardized data re-analysis was required so that all data matrices were scrutinized in a compatible and directly comparable way. The papers considered did not present all the same data analysis, particularly with regards to the inferences of historical demography. Moreover, geographical partitions of samples were not always the same as intended for the analysis of the Atlantic–Mediterranean divide. We determined evolutionary models for each data set, so that AMOVA analysis could be performed under the most optimal assumptions.

Genetic diversity and population divergence

Evolution models for each species were determined by the software package MODELTETST 3.06 (Posada & Crandall 1998). However, as the most inclusive evolution model implemented in the software package ARLEQUIN 3.01 (Excoffier et al. 2005) is the Tamura–Nei model with the inclusion of gamma value (Tamura & Nei 1993), more complex models than this were downgraded to the Tamura–Nei model. To perform a hierarchical analysis of molecular variance (AMOVA, Excoffier et al. 1992), populations were grouped in a hierarchical decreasing spatial scale when significant genetic differences of the established partitions were detected. Groups were organized as follows: (i) group one, all populations as one single panmictic population; (ii) group two, Atlantic Ocean vs. Mediterranean Sea; and (iii) group three, Atlantic vs. Western Mediterranean vs. Eastern Mediterranean. Permutation procedures were used to construct null distributions and test the significance of variance components for each hierarchical comparison (Guo & Thompson 1992).

Demographic history

Mismatch analysis of sequences (frequency of pairwise differences between haplotypes) was carried out to explore the demographic history of the populations studied. The method is based on the assumption that population growth or decline leaves distinctive signatures in the DNA sequences compared with a constant population size. Recent growth is expected to generate a unimodal distribution of pairwise differences between sequences (Rogers & Harpending 1992). The distribution is compared with that expected under a model of population expansion (Rogers 1995), calculating the estimator expansion time, τ (τ) and the mutation parameter (θ) according to Schneider & Excoffier.
The formula \( t = \frac{\tau}{2\mu k} \) was used to estimate the timing of population expansions (t), where \( \mu \) is the mutation rate per site per year and \( k \) is the sequence length. Mutation rates were those proposed for each species in the original papers (summarized in Table S3, Supplementary material).

Tajima’s \( D \) statistic (Tajima 1989) and Fu’s \( Fs \) test (Fu & Li 1993; Fu 1997) for selective neutrality were calculated by arlequin. For neutral markers, these tests can be used to detect changes in population size. Significant negative \( D \) and \( Fs \) values can be interpreted as signatures of population expansion. The neutrality tests used may not have sufficient statistical power to detect population growth under certain circumstances (Ramos-Onsins & Rozas 2002), and the authors have chosen to use four mismatch curve patterns (top of Fig. 3) to classify signatures of demographic history of significant geographical partitions. In one case, bimodality was due to extremely far apart peaks (Dentex dentex), in those situations the authors decided to consider only the first peak.
Results of the meta-analysis

Genetic analysis of the geographical partitioning by species revealed four types of structure: panmixis (Balaeoptera physalus, Diplodus sargus, Pagellus bogaraveo, Pagrus pagrus and Scomber japonicus), Atlantic–Mediterranean geographical partitioning (Dentex dentex, Dicentrarchus labrax, Diplodus puntazzo, Hydrobia acuta, Lithognathus mormyrus, Meganyctiphanes norvegica, Pomatoschistus microps, P. minutus, Spondyliosoma cantharus and Tursiops truncatus) with a possible further subdivision between East and West Mediterranean (Sagitta setosa) and structures other than panmixis and Atlantic–Mediterranean geographical partitioning (Anguilla anguilla, Sardina pilchardus and Scomber scombrus) (Table S1, Supplementary material).

We inferred the historical demography pattern of each species from the observed mismatch distribution in combination with Tajima’s and Fu’s statistical tests for neutrality, the sum of squared deviations (SSD) test and the raggedness index (Table S2, Supplementary material). Mismatch analysis of the 32 data sets resulting from the geographical partition of the 20 selected species (for details see Table S2) exposed several patterns of frequency distributions (Fig. 3).

Most frequency distributions are classified into four types. Type A (nine cases) has a unimodal distribution, associated with a sudden population expansion; the mean moves further away from the y-axis with time since expansion (e.g. Sa. pilchardus, Sc. japonicus and Sc. scombrus). Type B (eight cases) has a negative binomial curve, which provides evidence for a very recent bottleneck or expansion (e.g. D. labrax, M. norvegica). Type C (six cases) represents a bimodal frequency distribution, which may be interpreted as evidence for population stasis (Zlojutro et al. 2006). However, in some instances, the bimodal distribution points to the presence of two distinct phylogroups (e.g. Alvarado-Bremer et al. 2005). In our study, inspection of the haplotype networks (not shown) suggested that in all cases the two lineages are more or less differentiated from each other. The most recent mode depicts intraspecific pairwise differences and the second mode may point to more ancestral and emerging interspecific differences (e.g. De. dentex Atlantic and H. acuta Mediterranean). Finally, type D (nine cases) has a ragged distribution, associated with stable size populations (e.g. Tur. truncatus and Dip. puntazzo-Atlantic).

The combined interpretation of all demographic indicators evidenced that 20 cases out of 32 could be considered as populations which experiences an expansion (Table S2).

The neutrality tests yield a range of patterns in the investigated samples. All Tajima’s D values were negative but only 14 out of 32 showed significant departures from neutrality. The Fu’s Fs test turned out to be much more sensitive to detect departures from mutation-drift equilibrium, as 20 Fs values were significant in the same set of 32 samples. The two tests were congruent in detecting significant departures from neutrality in only 13 cases; correlation between the two values is in fact weak (r = 0.583). There was no general trend as in some instances, Mediterranean populations did not appear in equilibrium when compared to the Atlantic populations (e.g. De. dentex and Dip. puntazzo). In other species the opposite was true, such as for M. norvegica, the northern krill, and the two species of gobies (Pom. minutus and Pom. microps).

The timeframe analysis of the 20 sets used for qualifying an expansion comprised 16 species (13 fish species, one mollusk, one crustacean and one arrow worm) (Tables S2 and S3). Substitution rates varied from 0.94% to 3.05% million years (Myr) (Van Houdt et al. 2003) for fish cytochrome b, 2%/Myr (standard mitochondrial clock) (Brown et al. 1979) and 11%/Myr (Bargelloni et al. 2003) for fish control region, 2%/Myr and 193.3%/Myr (Peijnenburg et al. 2004) for Sa. pilchardus, 0.88% and 1.3%/Myr for M. norvegica (Papetti et al. 2005) and 1.62–2.04%/Myr (Wilke & Pfenninger 2002) for H. acuta. Tau values obtained from the mismatch analysis vary from 0.53 (P. bogaraveo) to 7.56 (Sc. japonicus). Average expansion values vary from 0.08 (Pom. minutus) to 1.62 Ma (De. dentex). Remarkably, in all cases, population expansion was calculated to be no older than the Pleistocene, a period characterized by strong climate cycling (Fig. 4).

The biological and genetic features of the 20 species were investigated using principal component analysis (PCA) (StatSoft Inc. 2001), which provides an alternative to reduce large data sets and search for an association among taxa on the basis of a certain number of characteristics. We took into
account both biological and genetic features. Biological characteristics (retrieved from the databank Fishbase, Froese & Pauly 2006) were: range of distribution both horizontal (geographical) and vertical (depth), lifestyle (pelagic, demersal, benthic), age at first maturity (years), generation time (years), trophic niche, sex (separate sexes, hermaphroditic-proterandric, hermaphroditic—proterogynic), type of fertilization (internal or external) and type of eggs (dispersal ability). Genetic measures were based on the re-calculated genetic data and included: AMOVA, percentage of genetic variation, mean mismatch value, $\tau$, $\theta_0$, $\theta_1$, Tajima’s $D$ and Fu’s $Fs$ tests.

The eigenvalues of the PCA corresponding to the first two principal components were 3.203 and 1.709, explaining 40.03% and 21.37%, respectively, of the total variability of the data. Exploration of the third principal component yielded an eigenvalue of 1.164% and 14.55% of additional variability. These results did not show any particular trend. In addition, there was no correlation between principal components and the set of biological and genetic variables considered.

What do we learn from the literature and data re-analysis?

A generalized picture of contrasting situations emerges, with cases of a lack of population structure, Atlantic—Mediterranean separation, and a population structure not associated with the Atlantic—Mediterranean partitioning. However, general trends and eventually, explanations for this apparent incongruence come from investigating the demography by mismatch analysis. Here, pairwise differences in haplotype sequence are tallied and compared to a negative binomial distribution (pointing to a stable or even decreasing population) and a unimodal Poisson distribution, pointing to a single or multiple expansions. Signatures of a bottleneck followed by population expansion seem associated with little or no population structure. *Sardina pilchardus, Scomber japonicus* and *Diplodus sargus* were characterized by the absence of geographical differentiation across the Atlantic—Mediterranean range. Interestingly, they showed a unimodal curve, typical of a population expansion. Extinction/recolonization may therefore justify the shallow population structure observed in these species. On the other hand, *Diplodus puntazzo* and *Hydrobia acuta*, differentiated between Atlantic and Mediterranean populations, were characterized by multiple population expansions (i.e. bimodal mismatch distribution) and mutation—drift equilibrium. One could generalize that species, which experienced expansion, after a (likely) drastic reduction of the effective population size erased signals of a historical population structure. Therefore, the more recent the bottleneck/expansion, the less time has passed to accumulate differences, even in the complete absence of gene flow.

Mitochondrial genes provide an advantage at inferring the history of the species, provided that a plausible evolutionary model for the gene is available (Emerson et al. 2001). However, particularly with mitochondrial genes, it is difficult to disentangle historical signals from present-day patterns of gene flow, as the signature of historical processes might obscure the current patterns of intraspecific processes. Low levels of mitochondrial genetic diversity might also result from genetic sweeps and not only from demographic histories. Lack of recombination in fact, makes this molecule prone to ‘genetic hitch-hiking’ that may erase historical signals (Bazin et al. 2006). Nevertheless, the extent of this genetic sweep seems to be dependent on the population size of the species (Mulligan et al. 2006); it is difficult to predict its impact on marine species with their large census population sizes and much smaller effective population sizes. Microsatellites, on the other hand, are believed to be more accurate in describing contemporaneous genetic relationships among populations and prove to be very sensitive to subtle genetic differences even over limited geographical scales (Zane et al. 2002). An important point of reference is the timing of a phenomenon. Time of population expansion as well as time of separation between divergent lineages (i.e. Mediterranean and Atlantic clades) can be estimated if a suitable mutation rate is assumed for the gene under investigation. In most vertebrate studies, the same mitochondrial locus (control region) was used for the analysis of population dynamics. Therefore, even when using inappropriate mutation rates for the D-loop, results should be comparable. The more accurate the placement of evolutionary processes such as bottleneck/expansion or vicariant separation between lineages into the appropriate timeframe, the better our ability to match them with the geological history of the area of interest. The ultimate goal is to identify along with the history of a region, geological and palaeo-oceanographical events that might have promoted evolutionary patterns shared by groups of species.

The phylogeographical patterns obtained from the data re-analysis seem at first inspection very diversified, with few indications for general conclusions. Such results are not unexpected given the diversity of the geographical regions considered, the tormented geological and climatic history of the area and the wide range of life-history traits of the taxa concerned. However, some generalities appear obvious:

- Three general structures of phylogeography are prominent: (i) Full congruence between Atlantic and Mediterranean clades (panmixia, e.g. *Sc. japonicus* — the chub mackerel, key speciea in pelagic ecology) suggests that gene flow between both seas is high, which is an intrinsic feature of fully pelagic species. However, this potential is not necessarily realized as the eutrophic Atlantic shelf and the oligotrophic Mediterranean Sea have
Genetic diversity does not necessarily decrease in a population if environmental suitability decreases. This might be the case for the bluefin tuna (*Thunnus thynnus*) and some sparid species (*Dip. sargus*, *Pagellus bogaraveo* and *Pagrus pagrus*). 

Several causes can be put forward: historic separation, temperature fluctuations (Aksu et al. 2002), plankton concentration and larval retention. Each of these factors underwent wide fluctuations over time into the Mediterranean Sea, particularly during the Quaternary age (Krom et al. 1999).

In parallel with microfossil evidence (Lambeck et al. 2002), divergence between older marine clades coincides with the first cooling down of the region during the period 3.2–1.8 Ma. Three major timings of the expansion patterns seem relevant: a Late-Pleistocene expansion [*A. anguilla*, *Dip. sargus*, *H. acuta* (Mediterranean), *L. mormyrus* (Mediterranean), *M. norvegica* (Atlantic), *P. bogaraveo*, *Pom. microps* (Mediterranean) and *Pom. minutus* (Atlantic)], Mid-Pleistocene expansion [*De. dentex*, *L. mormyrus* (Atlantic), *Pa. pagrus*, *S. scombrus*, *S. cantharus*, *L. budegassa*] and an Early Pleistocene expansion [*Dip. puntazzo* (Mediterranean), *S. setosa*, *S. pilchardus*, *Sc. japonica*, *Lo. piscatorius*]. The question remains why each species only expanded during those time periods. The most appropriate answer would come from an analysis of intrageneric species pairs such as *Dip. puntazzo*–*Dip. sargus*, *S. scombrus*–*S. japonicus* and *Lo. budegassa–L. piscatorius* (see above). More than the anticipated biological similarity between pairs, dissimilarities in phylogeographical pattern and population dynamics/history seems to be the more common theme. Divergence does not seem to be directly attributable to life history, reproduction, ecological niche or other biological traits. This might argue that organismal determinism may play a far less significant role than marine biogeographers have generally believed. As Cunningham & Collins (1998) noted, it is possible that taxa share histories simply because there are a limited number of possible histories, and for purely stochastic reasons species will share them.

Species in the Mediterranean Sea do not show a uniform phylogeographical pattern. Most striking is the impact of any combination of two extreme cases: complete genetic separation between Atlantic–Mediterranean populations since the early Pliocene and complete absence of population differentiation, usually following late Pleistocene recolonization. Ideally, the phylogeographical data would match with the detailed Pleistocene micropalaeontological record. Unfortunately, our phylogeographical insights do not reach the same level of resolution. We are not yet able to provide a genome-based reconstruction of the palaeosystem, although we are moving in this direction. We do not fully grasp the role of species-specific biological traits in determining the patterns observed. Moreover, the general picture lacks information from the southern continental shelf and offshore. What is needed is a more extensive sampling coverage as well as an improved phylogeographical calibration. These represent our challenge for the future.
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References


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Supplementary material

The following supplementary material is available for this article:

**Table S1** Information on the species data sets analysed (reference, sample size and location), genetic parameters (number of nucleotides, polymorphic sites and haplotypes) main genetic partition results (genetic pool, genetic variation and genetic partitioning) and demographic parameters (values of mismatch observed mean, tau τ, theta, q₀ and q₁) (A), Significant gene pools: 0, no panmixia, but structure not associated with Atlantic–Mediterranean differentiation; 1, one gene pool; 2, Atlantic and Mediterranean; 3, Atlantic, Western Mediterranean and Eastern Mediterranean. (B), Percentage of genetic variation (from AMOVA): if gene pool < 1, source of variation, among populations; if gene pool = 2, source of variation, among groups. (C), Partition of samples: 0, one gene pool; 1, Atlantic; 2, Mediterranean; 2’, Western Mediterranean; 2″, Eastern Mediterranean. (D), No samples from Eastern Atlantic, so only two gene pool arrangement could be tested. (E), Samples from Venice not included as there is the suspicion of another taxon being involved. (F), Samples from Black Sea not included

**Table S2** Species and geographical partitions used in the historical demography analysis. Data sets (8) refer to the last column of Table 1, partitions refer to Table 1 of the supplementary material, graph type relates to Figure 3. In bold significant results at the 0.05 level, except Fu’s Fs test, which was tested at the 0.02 level

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