Genetic differentiation between eastern and western Mediterranean swordfish revealed by phylogeographic analysis of the mitochondrial DNA control region

Jordi Viñas, Alexandra Pérez-Serra, Oriol Vidal, Jaime R. Alvarado Bremer, and Carles Pla


Despite there being evidence of several discrete breeding grounds, Mediterranean populations of swordfish have been considered a single panmictic unit with no genetic substructure. Sequence analysis of the mitochondrial DNA (mtDNA) control region of five Mediterranean locations (n = 251) reveals for the first time a clear genetic differentiation between eastern and western Mediterranean populations. This differentiation was detected only after conducting separate phylogeographic analyses on two previously described mtDNA clades. Although the frequencies of these clades are similar throughout the Mediterranean Sea, the levels of intra-clade genetic variation drop substantially towards the eastern end. This, together with clear differences in past demographic history and uneven migration rates between Mediterranean basins, suggests that the two populations experienced different effects during the Pleistocene. Subsequently, the mtDNA distinctiveness of eastern and western Mediterranean swordfish populations has been maintained probably by homing towards breeding areas.

Keywords: Mediterranean Sea, migration rates, past demographic analysis, phylogeography, stock structure, swordfish, Xiphias gladius.

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Introduction

Marine fish show little intraspecific population heterogeneity (Ward et al., 1994), the result of the absence of conspicuous geographic barriers in the marine realm, meaning that the dispersal capability of species facilitates high levels of gene flow (Palumbi, 1994). Additionally, many marine species tend to exhibit large population sizes that reduce the effects of genetic drift and, hence, increase the coalescent times of the alleles (Tajima, 1989). Contrary to these expectations, phylogeographic studies of several pelagic species have rejected the null hypothesis of panmixia, with population structure detected even on relatively small spatial scales (Magoulas et al., 1996, 2006; Viñas et al., 2004a; Patarnello et al., 2007). The origin of such differentiation has been associated with past biogeographic events affecting the demographic history of ancestral populations (Viñas et al., 2004a; Ely et al., 2005; Grant, 2005) and the vicariance–dispersal balance (Stepien and Rosenblatt, 1996). Current ecological biogeographic factors, such as dispersal potential, spawning behaviour, and population size, may maintain, or even reinforce, genetic differentiation between populations (Alvarado Bremer et al., 1996, 1998; Chow et al., 2000; Zardoya et al., 2004; Ely et al., 2005; Martinez et al., 2006). Therefore, unravelling the phylogeographic history of a species through assessing the genetic structure of its populations is a key factor for establishing appropriate management regulations for fisheries stocks (Pauly et al., 2002).

The swordfish (Xiphias gladius) is a large migratory fish found in the open waters of all oceans, including the Mediterranean Sea (Palomo et al., 2011). Although it is capable of long-distance migrations, several distinct populations both within and between oceans have been detected (Alvarado Bremer et al., 1995, 1996, 2005a; Rosel and Block, 1996; Chow et al., 1997; Chow and Takeyama, 2000). Currently, the International Commission for the Conservation of Atlantic Tuna (ICCAT) considers swordfish populations in the North Atlantic and Mediterranean Sea to be two separate stocks, so independent assessments and management recommendations are proposed for each area (Anon., 2003). These separate assessment–management units are supported in part by evidence from tagging programmes that has reported only a single recapture in the northeast Atlantic of a swordfish tagged in the western Mediterranean (Mejuto and Hoey, 1991; Jones, 1997; de la Serna et al., 2007). The presence of geographically discrete spawning areas also supports a two-stock hypothesis. Swordfish in the Atlantic reproduce across a wide band located in the western tropical area, with seasonal or year-round spawning that depends on oceanographic conditions (Mejuto, 2007). In the Mediterranean, spawning appears to be from June to August, when water temperatures and conditions are appropriate (Palomo et al., 2011; Rey, 1988). Within the Mediterranean, swordfish spawn in several discrete areas at the western (Rey, 1988; Di Natale et al., 2002) and eastern (Tserpes et al., 2001) ends.
Genetic studies on the population structure of swordfish have confirmed genetic differentiation between North Atlantic and Mediterranean populations, using sequence data from the mitochondrial DNA (mtDNA) control region (CR; Alvarado Bremer et al., 1995). The differentiation has subsequently been confirmed by studies using both mtDNA and nuclear markers (Kotoulas et al., 1995; Rosel and Block, 1996; Chow et al., 1997; Chow and Takeyama, 2000). Specifically, Alvarado Bremer et al. (1996) argued that the heterogeneity between the North Atlantic and Mediterranean populations was attributable to differences in the frequencies of two highly divergent mtDNA clades. Clade II haplotypes are absent from the Indo-Pacific, are found at low frequency in the South Atlantic (4%), increase in abundance in the North Atlantic (19%), and reach the greatest frequencies in the Mediterranean (38%). Based on these phylogeographic associations, Alvarado Bremer et al. (2005b) suggested that Clade I originated in the Pacific and Clade II in the Atlantic. In the last study, a subdivision within Clade II was also identified, with the reciprocal monophyletic association of subclades to the Atlantic and Mediterranean, respectively, corroborating the genetic isolation of Mediterranean swordfish from the Atlantic population.

Currently, all Mediterranean swordfish are considered to constitute a single population and are managed as one stock (Anon., 2003), previous genetic studies not having provided evidence to refute this hypothesis. Specifically, no differences in the distribution of haplotypes from three Mediterranean locations and one next to the Strait of Gibraltar, in the Gulf of Cádiz in the Atlantic Ocean, were revealed by RFLP (Restriction Fragment Length Polymorphism) of the entire mtDNA genome (Kotoulas et al., 1995). Also, no differentiation between Mediterranean and Gulf of Cádiz samples was found by analysing data from either the nuclear calmodulin gene or with RFLP of the mtDNA CR (Chow and Takeyama, 2000). Similarly, comparison of material from five Mediterranean locations and one from the Gulf of Cádiz using allozyme data led Pujolar et al. (2002) to reach similar conclusions. A more recent study that attempted to elucidate the phylogeographic history of Mediterranean swordfish (Alvarado Bremer et al., 2005b) failed to find genetic substructuring within the Mediterranean Sea. Hence, under the assumption of genetic homogeneity, Alvarado Bremer et al. (2005b) postulated that most of the mtDNA genetic variability in Mediterranean swordfish could be explained by four clusters of sequences whose members were closely related to four haplotypes present at high frequency. Moreover, the origin of these sequence groups could be traced to several invasions of swordfish from the Atlantic and from the Pacific via the Atlantic during Pleistocene interglacials, followed by isolation during glacial periods.

Despite the apparent genetic homogeneity of swordfish in the Mediterranean, some evidence does suggest population substructuring. In an allozyme analysis, a neighbour-joining tree clustered samples from eastern locations on a branch with 100% bootstrap support, although exact tests for genetic differentiation were not significant (Pujolar et al., 2002). Furthermore, the presence of discrete spawning areas in each basin could support a hypothesis of population subdivision. Therefore, we decided to test, using a phylogeographic approach, the null hypothesis of a single panmictic population of swordfish within the Mediterranean. An alternative hypothesis would be that swordfish populations in the western and eastern basins are genetically substructured. To discriminate between these hypotheses, we analysed the sequence variability in the hypervariable mtDNA CR-I in swordfish from five locations, two in the eastern basin and three in the western.

### Material and methods

Genetic analyses were conducted on 251 swordfish collected from three western Mediterranean locations, the Balearic Sea (n = 33), the Tunisian coast, (n = 12), and the Ligurian Sea (n = 59), and from two eastern Mediterranean locations, the Ionian Sea (n = 77) and the Aegean Sea (n = 70). The dataset included sequences of Mediterranean swordfish from a previous study (that of Alvarado Bremer et al., 2005b), which were combined with 73 new sequences. Sample details are included in Table 1. Skeletal muscle from each fish was collected and preserved in 96% ethanol or frozen at −30°C until laboratory analysis. Methods for DNA isolation, PCR amplification, and DNA sequencing followed Viñas et al. (2004b). Sequences of haplotypes were submitted to GenBank and assigned accession numbers EU827744–EU827798 (see Supplementary Table S1 for a summary of the material).

Sequences were aligned in BIOEDIT (Hall, 1999) and optimized by eye. The best-fit model of evolution was identified using the hierarchical series of likelihood ratio tests implemented in MODELTEST v 3.7 (Posada and Crandal, 1998). The most appropriate model was the HKY + G (Hasegawa et al., 1985) model with α = 0.312, transversion/transition ratio of 1.945, and base frequency of A: 0.342, C: 0.217, G: 0.156, and T: 0.284. Once all the haplotypes were identified, haplotypic (h; Nei and Tajima, 1981) and nucleotide diversities (π; Nei, 1987) and mean sequence divergence between groups corrected by within-group divergence (Dxy; Nei and Tajima, 1981) were computed in ARLEQUIN v 3.11 (Excoffier et al., 2005), using a distance matrix of HKY + G.

An analysis of molecular variance, AMOVA (Excoffier et al., 1992), was used to test several hypotheses of population structure, including genetic differentiation between the eastern and the western Mediterranean swordfish incorporating all lineages (pooled Clades I and II), and for each clade separately. The significance levels were determined by 10 000 permutations of samples in ARLEQUIN. Median joining (MJ) networks (Bandelt et al., 1999) for the entire dataset were built using the software NETWORK v 4.510, using the default settings.

Demographic history was inferred using two approaches. First, deviation from neutrality, expected following a population expansion, was evaluated using Tajima’s D (Tajima, 1989) and R2 (Ramos-Onsins and Rozas, 2002). The significances of these statistics were estimated using DnSP 4.00 (Rozas et al., 2003) with 1000 simulated resampling replicates. Alternatively, a population

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>M</th>
<th>Coordinates</th>
<th>SL (s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Mediterranean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ligurian Sea</td>
<td>59</td>
<td>34</td>
<td>43°30’N 9°E</td>
<td>110.6 (25.4)</td>
</tr>
<tr>
<td>Balearic Sea</td>
<td>33</td>
<td>23</td>
<td>39°30’N 0°10’E</td>
<td>120.8 (40.6)</td>
</tr>
<tr>
<td>Tunisian coast</td>
<td>12</td>
<td>10</td>
<td>37° N 10°E</td>
<td>90.7 (37.8)</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aegean Sea</td>
<td>70</td>
<td>28</td>
<td>37° N 25°E</td>
<td>145.9 (28.8)</td>
</tr>
<tr>
<td>Ionian Sea</td>
<td>77</td>
<td>39</td>
<td>39°30’N 17°30’E</td>
<td>104.8 (34.3)</td>
</tr>
<tr>
<td>Pooled Mediterranean</td>
<td>251</td>
<td>96</td>
<td>–</td>
<td>114.6 (20.6)</td>
</tr>
</tbody>
</table>

### Table 1. Swordfish sampling details, showing the number of individuals (n) and haplotypes (M), the average standard length (SL), and the standard deviation (s.d.).
that has experienced a rapid expansion in the recent past is expected to show a smooth wave-like mismatch distribution with a star-like phylogeny (Slatkin and Hudson, 1991; Rogers and Harpending, 1992). Hence, mismatch distribution analyses (Rogers and Harpending, 1992; Rogers, 1995), under the assumption of selective neutrality, were also used to evaluate possible historical events of population growth. Demographic parameters, including $\pi$, $\theta_{05}$ and $\theta_{1}$, and their probabilities were estimated in ARLEQUIN, taking into account the heterogeneity of mutation rates.

Migration rates ($M$) and the population size parameter ($\theta$) were inferred using the maximum likelihood (ML) inference in MIGRATE v 3.0 (Beerli and Felsenstein, 1999, 2001) among genetically differentiated swordfish populations. $M$ and $\theta$ were calculated using $F_{ST}$ estimates and UPGMA as starting points, and taking into account the HKY + G model of evolution. A Markov Chain Monte Carlo was run for ten short chains and three long chains with 10 000 and 100 000 recorded genealogies, respectively, after discarding the first 10 000 genealogies. One of every 20 reconstructed genealogies was sampled for both short and long chains.

Results

Sequence comparison of 303 bp of the mtDNA CR-I from 251 fish revealed 112 polymorphic sites, 51 of which were parsimony informative. These polymorphisms defined 96 distinct haplotypes with an overall haplotypic diversity of $h = 0.948 \pm 0.007$ and a nucleotide diversity of $\pi = 0.047 \pm 0.024$. In accord with the results of previous studies, the mtDNA haplotypes of Mediterranean swordfish clustered into two highly divergent clades, Clade I and Clade II, with a corrected sequence divergence of $D_a = 4.2\%$ ($\pm 1.1\%$). The average haplotype frequencies for the pooled Mediterranean locations were 64% for Clade I and 34% for Clade II. The greatest frequency of Clade I (75%) was in the Balearic Sea, but it averaged ~63% in the remaining localities, except the Tunisian coast, where the two clades were in equal proportion (Figure 1). The low sample size ($n = 12$) of the Tunisian coast material may account for this departure from other Mediterranean localities. Nevertheless, there were no significant differences in Clade I and Clade II frequencies among locations ($\chi^2$ test, $p = 0.385$).

A comparison among locations shows a decrease in the haplotypic diversity from west to east (Table 2, Figure 1). The largest values were from the Tunisian coast ($h = 0.970$) and the Balearic Sea ($h = 0.962$), lower in the central locations, i.e. the Ligurian Sea ($h = 0.955$) and the Ionian Sea ($h = 0.951$), and were lowest of all in the easternmost sample, the Aegean Sea ($h = 0.902$). All these values are substantially lower than the haplotypic diversity reported for the Northwest Atlantic ($h = 0.995$) by Alvarado Bremer et al. (2005b). In contrast, the levels of nucleotide diversity were similar throughout the Mediterranean (Table 2), and also similar to swordfish in the Northwest Atlantic (Figure 1).

This longitudinal cline in genetic variation became more apparent when data for two clades were analysed separately (Figure 1). The haplotypic diversity for Clade I decreased from $h = 0.957$ in the Balearic Sea to $h = 0.804$ in the Aegean Sea. Although the values of nucleotide diversity do not conform to the same pattern, they coincide in that the lowest value came from the Aegean Sea. The reduction in genetic variability from the western to the eastern Mediterranean becomes more apparent when Clade II data are analysed. Therefore, haplotypic diversity for Clade II sequences dropped almost 20% between the Balearic Sea ($h = 0.964$) and the two eastern basin locations, the Ionian Sea ($h = 0.794$) and the Aegean Sea ($h = 0.889$; Table 2, Figure 1). Similar to Clade I, the nucleotide diversities for Clade II were similar among locations, but also showed the lowest value in the Aegean Sea.

The AMOVA of the complete set of sequences failed to identify genetic differentiation among swordfish within the Mediterranean ($\Phi_{ST} = 0.009; p > 0.05$; Table 3). However, separate analyses of the two clades revealed a significant proportion of variation among regions (Clade I, $\Phi_{ST} = 0.058$, $p = 0.000$; Clade II,
Table 3. AMOVA results of Mediterranean swordfish populations.

<table>
<thead>
<tr>
<th>Structure tested</th>
<th>Variance component</th>
<th>Percentage of total</th>
<th>Fixation index ($\Phi_{ST}$)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole dataset, one gene pool (all locations)</td>
<td>0.063</td>
<td>0.99</td>
<td>0.009</td>
<td>0.156</td>
</tr>
<tr>
<td>Among regions</td>
<td>6.329</td>
<td>99.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within regions</td>
<td>3.249</td>
<td>94.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clade I, two gene pools (eastern vs. western Mediterranean)</td>
<td>0.203</td>
<td>5.89</td>
<td>0.058</td>
<td>0.000</td>
</tr>
<tr>
<td>Among regions</td>
<td>3.347</td>
<td>96.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within regions</td>
<td>1.191</td>
<td>96.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clade II, two gene pools (eastern vs. western Mediterranean)</td>
<td>0.045</td>
<td>3.67</td>
<td>0.037</td>
<td>0.006</td>
</tr>
<tr>
<td>Among regions</td>
<td>3.347</td>
<td>96.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within regions</td>
<td>1.044</td>
<td>97.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Probability of finding a more extreme variance component and $\Phi_{ST}$ index than observed by chance alone after 1000 permutations.

$\Phi_{ST} = 0.037, p = 0.006$; Table 3). Furthermore, the hypothesis of two separate genetic pools was tested by comparing the pooled data for the eastern Mediterranean with the pooled data for the western Mediterranean, by clade. In both instances, significant genetic differentiation was detected (Clade I, $\Phi_{ST} = 0.034, p = 0.009$; Clade II, $\Phi_{ST} = 0.026, p = 0.001$; Table 3).

The MJ network clearly identified the two swordfish mtDNA clades, interconnected by a long branch (Figure 2). Clade I included four centroids with counts of 13, 14, 19, and 36 fish (Supplementary Table S1). The centroid with 13 individuals was found only in the eastern Mediterranean, i.e. the Aegean and Ionian Seas. Clade II is characterized by a single star-like formation featuring one major centroid with a total of 33 individuals from both the eastern and the western Mediterranean locations.

Demographic history parameters were estimated separately for each clade by region, i.e. western and eastern Mediterranean (Table 4). Mismatch analyses for Clade I in both basins showed multimodal patterns not congruent with the sudden growth expansion model (Figure 3). Both Tajima’s $D$ and $R_2$ tests failed to reject neutrality for this clade in the two Mediterranean basins (Table 4). In contrast, significant departures from neutrality were observed for Clade II in both the western and the eastern Mediterranean (Table 4). These results, together with the bell-shaped mismatch distributions for Clade II sequences (Figure 3), indicate that this clade experienced sudden expansion in both regions. However, the $\tau$ estimate of expansion is larger in the eastern ($\tau = 2.031$) than in the western basin ($\tau = 1.410$), indicating a more recent population expansion event in the west. Although estimates of the magnitude of expansion (difference $\theta_0$ from $\theta_1$) are less reliable than estimates of the time of expansion (Schneider and Excoffier, 1999), the larger value of $\theta_1$ in the western basin may indicate that the expansion of Clade II in the western basin was larger than in the eastern basin (Table 4).

Migration rates and effective population size parameters of swordfish populations were estimated using MIGRATE, based on genetically differentiated populations revealed by AMOVA and for each mtDNA CR-I clade separately. The ML inferences produced stable results after the first run, and similar results were obtained with larger long and short chains, and even when ten replicates were tested (data not shown). Similar to the results for demographic history, the estimates of population size were larger in the western Mediterranean for both clades (Clade I, $\theta = 1.157$; Clade II, $\theta = 9.84 \times 10^{11}$) than in the eastern Mediterranean (Clade I, $\theta = 0.079$; Clade II, $\theta = 0.029$). The direction of migration was highly asymmetrical for the two clades (Figure 4), and in both instances, it was considerably greater from the east towards the west. The highest estimate of migration was for Clade I towards the west, with a substantially smaller number of migrants moving to the east. For Clade II, migration was exclusively westward, with the immigration rate into the eastern population approaching zero.

![Figure 2. MJ network for the complete dataset of Mediterranean swordfish. Black slices indicate western origin and white slices eastern origin.](https://academic.oup.com/icesjms/article-abstract/67/6/1222/737624/2.293 (0.005) 0.046 (0.001) 1.376 (0.054) 0.062 (0.156) 1.938 (0.007) 0.036 (0.006) 2.293 (0.005) 0.046 (0.001) 1.376 (0.054) 0.062 (0.156) 1.938 (0.007) 0.036 (0.006) 1.938 (0.007) 0.036 (0.006) 1.938 (0.007) 0.036 (0.006)

Table 4. Demographic parameters separately for the two mtDNA clades (Clade I and Clade II) for each genetically differentiated population (western, W, and eastern, E, Mediterranean basins), showing the number of individuals ($n$) and haplotypes ($M$), the haplotypic diversity ($h$), the nucleotide diversity ($\pi$), and the standard deviations (s.d.).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$n$</th>
<th>$M$</th>
<th>$h$ (s.d.)</th>
<th>$\pi$ (s.d.)</th>
<th>$\theta_M$, $\tau$, $\theta_1$</th>
<th>$D$ ($p$)</th>
<th>$R_2$ ($p$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clade I W</td>
<td>68</td>
<td>35</td>
<td>0.929 (0.020)</td>
<td>0.024 (0.012)</td>
<td>0.000, 10.242, 15.195</td>
<td>-0.674 (0.276)</td>
<td>0.752 (0.240)</td>
</tr>
<tr>
<td>Clade II W</td>
<td>36</td>
<td>20</td>
<td>0.894 (0.042)</td>
<td>0.008 (0.005)</td>
<td>0.000, 1.410, 99.999</td>
<td>-2.293 (0.005)</td>
<td>0.046 (0.001)</td>
</tr>
<tr>
<td>Clade I E</td>
<td>93</td>
<td>36</td>
<td>0.908 (0.018)</td>
<td>0.020 (0.011)</td>
<td>0.000, 10.191, 10.115</td>
<td>-1.376 (0.054)</td>
<td>0.062 (0.156)</td>
</tr>
<tr>
<td>Clade II E</td>
<td>54</td>
<td>19</td>
<td>0.813 (0.048)</td>
<td>0.006 (0.004)</td>
<td>0.000, 2.031, 10.045</td>
<td>-1.938 (0.007)</td>
<td>0.036 (0.006)</td>
</tr>
</tbody>
</table>

Also given are the values of Tajima’s $D$ and $R_2$ neutrality tests with probability values ($p$).
Discussion

Compared with Atlantic populations, Mediterranean swordfish populations have lower levels of mtDNA sequence variation (Figure 1). This reduction in genetic variation is manifest in consistently smaller values of haplotypic diversity in the Mediterranean [0.948 in this study, 0.942 in Alvarado Bremer et al. (2005b), 0.85 in Alvarado Bremer et al. (1995), 0.94 in Alvarado Bremer et al. (1996), and 0.832 in Rosel and Block (1996)] than in the Atlantic (>0.988). Interestingly, a clinal decrease in genetic variability was observed from west to east in the Mediterranean (Figure 1).

AMOVAs for the complete dataset failed to reject the hypothesis of a single genetic stock for swordfish in the Mediterranean Sea (Table 3). However, given that the two mitochondrial clades in swordfish have distinct phylogeographic origins (Alvarado Bremer et al., 2005a), it is appropriate to analyse them separately (Alvarado Bremer et al., 2005b; Martinez et al., 2006). The separate AMOVA tests showed clear patterns of genetic differentiation between swordfish from the eastern and the western Mediterranean, for both clades (Table 3). Therefore, our results support the hypothesis of there being two genetically differentiated populations of swordfish in the Mediterranean, one in the western and another in the eastern basin.

Surprisingly, genetic differentiation for Mediterranean swordfish is not a consequence of the increase in the abundance of specimens with Clade II haplotypes, a set of lineages with consistently lower levels of genetic diversity (Table 2). Instead, there is a reduction in the genetic variability in both mtDNA clades from the western to the eastern Mediterranean (Figure 1). Clade frequencies are similar throughout the Mediterranean, 63 and 37% for Clade I and Clade II, respectively. This geographic pattern contrasts with the pattern observed in other phylogeographic studies of swordfish in which genetic differentiation, at both inter- and intra-oceanic scales, has been attributed to heterogeneity of the frequency distributions of the two mtDNA clades (Alvarado Bremer et al., 1996; Rosel and Block, 1996). Shifts in the frequency of clades have been reported for several other pelagic species at an interocean scale (Finnerty and Block, 1992; Graves and McDowell, 1995; Alvarado Bremer et al., 1998; Chow et al., 2000; Martinez et al., 2006). Similarly, in the Mediterranean, geographic variability in the mtDNA clade frequencies, but not reduction in intra-clade genetic variability, has resulted in genetic heterogeneity among anchovy (Engraulis encrasicolus) populations (Magoulas et al., 1996, 2006; Grant, 2005) and in the isolation by distance (IBD) reported in Atlantic bonito (Sarda sarda; Viñas et al., 2004a).

Rejection of a model of panmixia in a relatively small sea basin for a large pelagic species such as swordfish was unexpected. The result differs from previous studies surveying allozymes (Pujolar et al., 2002), RFLPs of the entire mitochondrial genome (Kotoulas et al., 1995), analyses of a single-copy nuclear
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Calmodulin gene, and PCR–RFLP data of the mtDNA CR (Chow and Takeyama, 2000). Note, however, that evidence of genetic differentiation between eastern and western Mediterranean swordfish is apparent after reinspecting the allozyme data of Pujolar et al. (2002). In the neighbour-joining tree (Figure 1 in Pujolar et al., 2002), the two samples from eastern locations (Aegean and Ionian) cluster together and are separated from the western Mediterranean locations by a branch supported by a high bootstrap value (90%). This pattern of differentiation was attributed by those authors to the greater frequency of isocitrate dehydrogenase (IDPH-2⁺) allele 70 in samples from the east, although exact probability tests of differences among populations were not significant. In contrast, the results of the present study using a hypervariable marker combined with a phylogeographic analysis reveals previously hidden genetic heterogeneity in the distribution of variation within a relatively small region such as the Mediterranean Sea. A similar result, also combining phylogeographic and historical demography analyses, revealed genetic heterogeneity among Atlantic bonito populations in the Mediterranean using CR data (Viiñas et al., 2004a). In that instance, a clade conforming to IBD was documented, revealing a pattern of genetic variation that could not be detected using allozymes or an RFLP analysis of the entire mtDNA molecule.

Two alternative, although not exclusive, hypotheses based on present-day life-history traits may explain the observed heterogeneity among populations of swordfish. First, a larval retention mechanism (Iles and Sinclair, 1982) may prevent gene flow between populations in the two Mediterranean basins. Surface currents in the eastern Mediterranean flow anticyclonically and are complicated by several eddies and jets (Hamad et al., 2005). The eastern basin has greater water transparency, higher salinity, and is more oligotrophic as a consequence of the inflow of ultraoligotrophic water through the Bosphorus from the Black Sea, and intense evaporation, in contrast to the less dense water in the western basin from the Atlantic (Krom et al., 2005). Oceanographic conditions were also invoked by Magoulas et al. (1996) to account for the observed patterns of differentiation in European anchovy, particularly with regard to the distinctiveness of the Aegean and Adriatic subpopulations. Alternatively, spawning site fidelity to discrete spawning grounds may also produce genetic heterogeneity. Within the Mediterranean, there is evidence of more than one swordfish spawning area, one being the Straits of Sicily, where swordfish eggs, larvae, and mature adults have been recorded (Rey, 1988; Anon., 2003). A second spawning area has been located in the Balearic Sea in the western Mediterranean by the presence of larvae and adults with large gonadosomatic indices (Di Natale et al., 2002). Additionally, mature females, linked to an abundance of males, were found in the Levantine Sea in the eastern Mediterranean by Tserpes et al. (2001). If spawning areas are discontinuous, then homing to breeding areas located in the eastern and the western basins could account for the Mediterranean population substructuring detected here. Spawning site fidelity has been invoked traditionally to explain genetic differences among swordfish populations at an interoceanic scale (Rosel and Block, 1996), but also at a large oceanic scale to account for the heterogeneity among Pacific swordfish populations (Alvarado Bremer et al., 2006). A recent comparison of North and South Atlantic swordfish populations is congruent with strong site fidelity (Alvarado Bremer et al., 2005a). Fish associated with spawning and feeding areas in the North and the South Atlantic were respectively more closely related to each

other than to any other region, although the spawning and feeding areas were separated by an average distance of 2500 km, a distance similar to distances between the eastern and the western breeding areas in the Mediterranean.

The genetic differentiation between western and eastern Mediterranean populations detected here gives rise to a new scenario that better resolves the phylogeographic history of the species. Recently, Alvarado Bremer et al. (2005b) hypothesized that the two mtDNA clades originated as a consequence of the rise of the Isthmus of Panama, ~3.5 million years ago, and not as a result of the vicariant isolation of the Mediterranean from the North Atlantic ~200 000 years ago, as suggested earlier in Alvarado Bremer et al. (1996). Further, the authors of the first-mentioned study observed that overall mtDNA genetic variation in the Mediterranean swordfish consisted of four star-like genealogies, three in Clade I and one in Clade II, probably a consequence of the same number of concurrent founder events by swordfish females with mtDNA lineages of Atlantic and Pacific ancestral origin. Although these differentiated groups of sequences appear in the Mediterranean haplotype MJ network (Figure 2), inspection of mismatch distributions and MIGRATE results suggests distinct phylogeographic histories in the western and the eastern Mediterranean basins, influenced by both asynchronous population collapses and Atlantic–Pacific invasions. In contrast, the Atlantic population remained large and stable.

During the last glacial maximum, ~18 000–21 000 years ago, Mediterranean populations were probably restricted to the eastern basin, where summer temperatures (~20°C; Thiede, 1978) were high enough for reproduction (Palko et al., 1981; Mejuto, 2007). With the arrival of warmer contemporary oceanographic conditions, the population expanded to the west. A reverse migration from the west to the east, particularly of individuals with Clade I, cannot be discarded (Figure 4), however, so populations in the western basin may have originated more recently and may have experienced greater population growth as a consequence of the rapid re-establishment of favourable oceanographic conditions fuelled by the inflow of Atlantic waters.

To summarize, this is the first study to provide evidence of genetic differentiation of swordfish within the Mediterranean. This population substructure is the consequence of distinct phylogeographic histories of populations in the eastern and the western Mediterranean basins and is maintained by present-day life-history traits, including homing fidelity to spawning sites. The analysis of mtDNA variability offers only a partial view of swordfish biology and population histories, because mtDNA is maternally inherited. Further studies with complementary genetic markers (i.e. nuclear markers), additional sampling, and other biological studies, including tagging experiments, are needed to corroborate the results presented here. However, the concordance of the results using nuclear DNA and mtDNA population markers regarding the differentiation between NW Atlantic, South Atlantic, Mediterranean, and Indo-Pacific swordfish populations suggests that mtDNA data alone may accurately reflect the population structure of the species. Therefore, based on these results, we would propose that the Mediterranean swordfish stock be managed as at least two distinct units: one in the eastern Mediterranean and another in the western Mediterranean. The current single-stock management unit approach used to manage Mediterranean swordfish by ICCAT should in our opinion be reconsidered, because management regulations aimed at large regional scales might overfish some small, but discrete populations.
Supplementary material

Supplementary material is available at ICESJMS online.

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References


Mejuto, J. 2007. Aspectos biológicos y pesqueros del pez espada (Xiphias gladius Linnaeus, 1758) del océano Atlántico, con especial referencia a las áreas de actividad de la flota española. Departamento de Biología animal, Universidad de Santiago de Compostela, Santiago de Compostela.


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