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Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea

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Abstract

Population genetic structures of the *mackerel (Scomber scombrus*) and chub mackerel (*Scomber japonicus*) were studied in the Mediterranean Sea. Fragments of 272 bp (*S. scomber*) and 387 bp (*S. japonicus*) of the 5′-end of the mitochondrial control region were sequenced from spawning individuals collected off the coasts of Greece, Italy, Spain, and Portugal. High levels of mitochondrial control region haplotypic diversity (> 0.98) were found for both Scomber species. Nucleotide diversity was higher in the mackerel (0.022) than in the chub mackerel (0.017). Global F_{ST} values were also higher and significant in the mackerel (0.024, $P < 0.0001$) as opposed to the chub mackerel (0.003, $P > 0.05$). Molecular variance analyses showed differential genetic structuring for these two closely related species. There is extensive gene flow between Mediterranean Sea and Atlantic Ocean populations of chub mackerel, which are organized into a larger panmictic unit. In contrast, Mediterranean Sea populations of mackerel show some degree of genetic differentiation and are structured along an east–west axis. The analysed eastern Mediterranean Sea mackerel populations (Greece, Italy) are clearly separated from that of the western Mediterranean Sea (Barcelona), which forms a panmictic unit with eastern Atlantic Ocean populations. The genetic structures of both species showed asymmetric migration patterns and indicated population expansion.

Keywords: chub mackerel, mackerel, marine speciation, mitochondrial control region, population genetics, Scomber

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Introduction

Marine pelagic and demersal fishes are expected to exhibit little intraspecific genetic structuring even over large geographic distances (Ward *et al*. 1994). Ocean currents and the apparent lack of physical barriers in the marine realm seem to greatly facilitate extensive gene flow among marine fish populations (Palumbi 1994). The potential for long distance dispersal and the large population sizes of both the larval and adult stages contribute to the low levels of genetic subdivision, eventually leading to panmictic populations (McQuinn 1997). Recent studies, however, challenge the long held view that recruitment in marine fish populations is independent of local reproduction, revealing cryptic population structuring (Miya & Nishida 1997; Rocha-Olivares *et al*. 1999; Nesbø *et al*. 2000; Hutchinson *et al*. 2001; Wirth & Bernatchez 2001; Knutsen *et al*. 2003). In the absence of physical barriers or great geographical distances, closely related marine fish species may exhibit population structuring based on subtle differences in spawning behaviour, self-recruitment, and mechanisms of near-shore retention of larvae (Knutsen *et al*. 2003). Hence, to better understand speciation mechanisms in the marine environment, it is mandatory to characterize not only population dynamics and structure, but also life-history strategies. At least three competing, but not exclusive,

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hypotheses may explain population structuring in marine pelagic and demersal fish species (Palumbi 1994; McLean *et al*. 1999; Avise 2000; Bahri-Sfar *et al*. 2000): (1) Environmental factors, including past sea level changes, and present or past physical barriers such as ocean currents, may mix or disrupt fish populations from different geographic locations; (2) increasing geographical distance is expected to enhance isolation among populations; (3) life-history traits, including potential for dispersal, homing to spawning zones, and larval retention, may play an important role in population structuring. Here, we examine the relative importance of these hypotheses in explaining marine population structure by comparing genetic differentiation in two Scomber species.

An accurate definition of population structure is particularly important for fisheries management of commercial marine fish (Utter 1991), since failure to detect population units can lead to local overfishing and ultimately to severe declines (Cook *et al*. 1997; Hutchings 2000; Knutsen *et al*. 2003). The mackerel (*Scomber scombrus* L.) and the chub mackerel (*Scomber japonicus* Houttuyn 1782) are two commercially important marine fish in the North Atlantic Ocean and the Mediterranean Sea (Scoles *et al*. 1998). Both species are pelagic, migratory and schooling, forming large shoals that in the case of the mackerel can reach millions of individuals (Lockwood 1988). Besides their economical value for European fisheries, both Scomber species occupy a key position in the trophic chain of eastern Atlantic Ocean and Mediterranean Sea ecosystems, as they are an essential element of the diet of larger pelagic fish (e.g. tuna, swordfish, and sharks) and sea mammals (e.g. dolphins and seals). Several studies on mackerel based on parasite infestation rates (Mackenzie 1983) and otolith morphology (Dawson 1986) have demonstrated the existence of three stocks (i.e. fish populations with a single spawning ground to which adults return every year) in the eastern Atlantic Ocean. Furthermore, tagging experiments have shown that all eastern Atlantic Ocean mackerel stocks gather in the North Sea after spawning (reviewed in Lockwood 1988). A recent comprehensive study based on mitochondrial (control region and cytochrome *b* gene) sequence data supported statistically significant population structuring among the three eastern Atlantic Ocean spawning stocks of mackerel, although no genetic differentiation was observed among shoals outside the spawning season (Nesbø *et al*. 2000).

In contrast to the wealth of information available for mackerel in the eastern Atlantic Ocean, there are only sporadic studies focused on Atlantic Ocean chub mackerel, and on the two Scomber species within the Mediterranean Sea (Lissner 1937; Andreu & Rodríguez-Roda 1953; Scoles *et al*. 1998; Roldan *et al*. 2000). The existence of an Atlantic Ocean-Mediterranean Sea transition (Borsa *et al*. 1997) was shown for many fish species, e.g. *Lithognathus mormyrus*, *Spondyliosoma cantharus*, *Dentex dentex* (Bargelloni *et al*. 2003), *Xiphias gladius* (Kotoulas *et al*. 1995), *Dicentrarchus labrax* (Naciri *et al*. 1999), *Trisopterus minutes* (Tirard *et al*. 1992), *Sardinella aurita* (Chikhi *et al*. 1997), *Mugil cephalus* (Crosetti *et al*. 1994). Moreover, an eastern-western Mediterranean Sea fragmentation around the Siculo-Tunisian strait was observed for the sea bass (*Dicentrarchus labrax* L.) (Barhi-Sfar *et al*. 2000). It is important to know whether such transition zones are also effective for Scomber species. Transition zones are expected to show a stronger effect of mechanisms that counteract gene flow and promote structuring (Barhi-Sfar *et al*. 2000). Hence, they are particularly interesting in discriminating among competing marine population structure hypotheses.

In this study, we investigated the genetic diversity of mackerel and chub mackerel in the Mediterranean Sea and South Iberian Atlantic Ocean waters. We used haplotype frequencies of mitochondrial control region sequence data because this genetic marker has proven to show adequate levels of sequence variation in the Atlantic Ocean mackerel (Nesbø *et al*. 2000). Our aim was to characterize population structuring of both of these commercially important species within the Mediterranean Sea and test whether the Mediterranean Sea populations are effectively distinct from the Atlantic Ocean ones. The long-term objective of this study was to infer the relative role of environmental factors and life-history strategies in shaping and maintaining the patterns of population structure of the two closely related species.

Materials and methods

Sampling

Samples were collected by purse seines from five well defined and separate areas of the Mediterranean Sea (Fig. 1), i.e. Thracian Sea (Kavala, Greece), southern Adriatic Sea (Bari, Italy), Siculo-Tunisian strait (Lampedusa, Italy), Thyrrenean Sea (Messina, Italy), and Catalan sea (Barcelona, Spain), as well as from the adjacent Atlantic Ocean waters, i.e. Algarve coast (Olhão, Portugal). The number of samples collected per site is listed in Table 1. Because Scomber species form mixed populations outside the spawning season (Nesbø *et al*. 2000), sampling was concentrated on spawning individuals. The sexual cycle of reproductive adults was determined by macroscopic examination of their gonads, and by calculating the gonado-somatic index [GSI; (gonad weight/eviscerated weight) × 100]. Only individuals at the first stage of maturation, in advanced maturation, or with ripe gonads were analysed. For *S. scombrus*, the spawning period was found to be in February in Greece and Italy, and in January in Spain and Portugal. For *S. japonicus*, the spawning period extended from April to August in the different locations, and the peak was achieved in June in Greece, in May in Italy, in August in Spain, and in April in Portugal. All samples were stored in 100% ethanol.

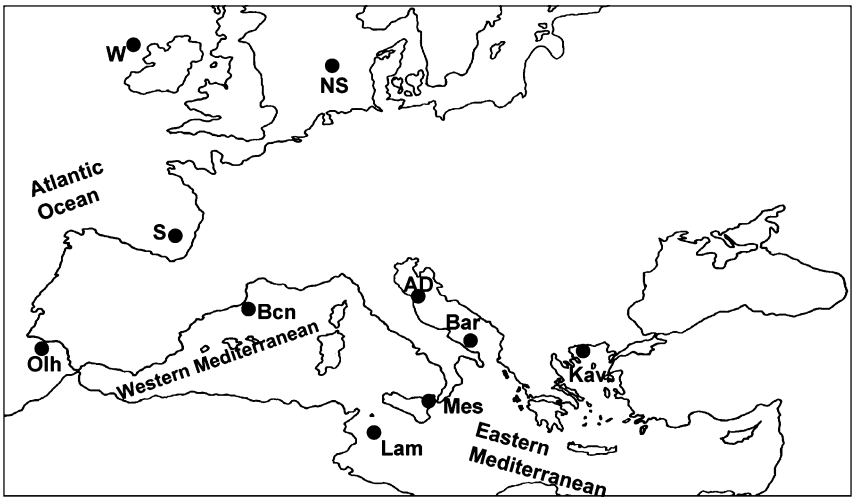

Fig. 1 Map showing the sampling locations of *Scomber scombrus* and *S. japonicus*. Abbreviations as follows: Olh — Olhão (Portugal); Bcn — Barcelona (Spain); Bar — Bari (Italy); Mes — Messina (Italy); Lam — Lampedusa (Italy); Kav — Kavala (Greece); from Nesbø *et al*. (2000), NS — Northern Sea; W — Western; S — Southern; AD — Adriatic (Ancona). Note: A sample from eastern Canada, from Nesbø *et al*. (2000), was also used in the analysis but not shown in the map.

Table 1 Descriptive statistics for the studied mackerel and chub mackerel populations*

Population	Sample code	Date	*n*	No. Haplotypes	*S*	H_{exp}	κ	π	θ	Reference
S. scombrus										
Kavala	Ss-Kav	Feb 01	50	34	46	0.96 ± 0.02	6.1 ± 3.0	0.020 ± 0.011	10.3 ± 3.3	Present study
Bari	Ss-Bar	Dec 00–Jan 01	48	31	43	0.93 ± 0.03	4.4 ± 2.2	0.015 ± 0.009	9.7 ± 3.0	Present study
Barcelona	Ss-Bcn	Dec 00	49	33	39	0.95 ± 0.02	5.7 ± 2.8	0.019 ± 0.011	8.7 ± 2.7	Present study
Olhão	Ss-Olh	Feb 01	49	42	58	0.99 ± 0.01	7.3 ± 3.8	0.024 ± 0.013	13.0 ± 3.9	Present study
Adriatic	AD	Jul 97	15	11	30	0.97†		0.026†		Nesbø *et al*. (2000)
Southern	S	Apr 97	17	14	29	1.00†		0.022†		Nesbø *et al*. (2000)
Western	W	May–Jun 97	22	12	23	0.95†		0.016†		Nesbø *et al*. (2000)
North Sea	NS	Jun–Jul 97	17	13	25	0.99†		0.016†		Nesbø *et al*. (2000)
Canada	Can	Jun 97	18	16	30	1.00†		0.028†		Nesbø *et al*. (2000)
S. japonicus										
Kavala	Sj-Kav	May–Jul 00	50	43	62	0.99 ± 0.01	8.3 ± 3.9	0.02 ± 0.01	13.8 ± 4.1	Present study
Lampedusa	Sj-Lam	May–Jun 00	27	26	37	1.00 ± 0.01	7.5 ± 3.6	0.02 ± 0.01	9.6 ± 3.3	Present study
Messina 1	Sj-Mes1	May 00	44	34	40	0.98 ± 0.01	6.1 ± 3.0	0.02 ± 0.01	9.2 ± 3.0	Present study
Messina 2	Sj-Mes2	Jun 00	44	38	42	0.99 ± 0.01	7.0 ± 3.4	0.02 ± 0.01	9.7 ± 3.1	Present study
Barcelona	Sj-Bcn	Aug 00	50	43	44	1.00 ± 0.01	7.2 ± 3.4	0.02 ± 0.01	9.8 ± 3.0	Present study
Olhão	Sj-Olh	Mar 01	48	39	45	0.98 ± 0.01	6.8 ± 3.3	0.02 ± 0.01	10.1 ± 3.1	Present study

*n = sample size, S = number of polymorphic sites, H_{exp} = gene diversity (Nei 1987), κ = mean pairwise nucleotide differences (Tajima 1993), π = nucleotide diversity (Nei 1987), θ = expected heterozygosity per site (Watterson 1975).
†taken from Table 2 of Nesbø *et al*. (2000).

DNA isolation, pcr amplification, and automated sequencing

Total DNA was extracted from muscle tissue or caudal fin clips. Each sample was homogenized overnight at 55 °C in extraction buffer (EDTA 0.1 M, Tris-HCl pH 8.0 0.05 M, SDS 1%, and Proteinase K 0.2 mg/mL). Subsequently, DNA was purified with a standard phenol/chloroform extraction protocol followed by an ethanol precipitation. The 5′-end of the mitochondrial control region of the Scomber specimens was amplified by polymerase chain reaction (PCR) (35 cycles of 1 min at 94 °C, 1 min at 50 °C and 1 min at 72 °C) using the L-Pro 1 (5′-ACTCTCACCCCTAGCTCCCAAAG-3′) and H-DL-1 (5′-CCTGAAGTAGGAACCAGATGCCAG-3′) primers (Ostellari *et al*. 1996). PCR fragments were purified by ethanol precipitation, and sequenced using the L-Pro 1 primer and the BigDye Terminator Sequencing Ready Reaction V. 3.0 kit (Applied Biosystems) following manufacturerís instructions in an ABI 3700 automated sequencer.

Sequence data analysis

Sixty-six different haplotypes (Table 1) corresponding to four Atlantic Ocean (Southern, Western, North Sea,

and Canada) and one Mediterranean Sea (central Adriatic, Ancona) *Scomber scombrus* populations (Fig. 1) were retrieved from http://biologi.uio.no/fellesavdelinger/dna-lab/d-loop.html (Nesbø *et al*. 2000) and analysed together with our 196 mackerel sequences.

Sequences were aligned using CLUSTAL X (Thompson *et al*. 1997) followed by refinement by eye. Gaps resulting from the alignment were treated as missing data. Phylogenetic relationships were estimated with PAUP* 4.0b10 (Swofford 1998) using the neighbour joining (NJ) method of phylogenetic inference (Saitou & Nei 1987). Genetic distances were corrected following the model of Tamura & Nei (1993), which was specifically conceived to reproduce the evolution of the mitochondrial control region. Among-site rate variation was corrected with the shape parameter of a gamma distribution, empirically determined from each NJ topology by maximum likelihood in PAUP*4.0b10 ($\square$ = 0.68 and 0.45 for *S. scombrus* and *S. japonicus*, respectively). The midpoint option was used for rooting the trees (high sequence divergence between species impeded the use of interspecific outgroups). Robustness of the resulting phylogenetic trees was tested by bootstrapping (Felsenstein 1985). In addition, genealogical relationships were examined based on reduced data sets that contained only the tranversions, by reconstructing haplotype networks using parsimony (Templeton *et al*. 1992) with TCS 1.03 (Clement *et al*. 2000).

Population genetic statistics were estimated using ARLEQUIN 2001 (Schneider *et al*. 2000). The level of polymorphism of each population was estimated as the number of polymorphic sites (S), gene diversity (H_{exp}; Nei 1987), the average number of pairwise nucleotide differences (κ; Tajima 1983), nucleotide diversity (π; Nei 1987) and expected heterozygosity per site (θ; Watterson 1975). Pairwise genetic divergences between populations were estimated using the fixation index Φ_{ST} (Excoffier *et al*. 1992), which includes information on mitochondrial haplotype frequency (Weir & Cockerham 1984) and genetic distances (Tamura-Nei with gamma correction). Significance of pairwise population comparisons was tested by 20 000 permutations. *P*-values were adjusted with the sequential Bonferroni correction (Rice 1989). Partitioning of genetic variability among populations was tested using a hierarchical analysis of molecular variance, AMOVA (Excoffier *et al*. 1992) with the software SAMOVA 1.0 (Dupanloup *et al*. 2002) that implements an approach to define groups of populations that are geographically homogeneous and maximally differentiated (Φ_{CT}) from each other. Permutation procedures (N = 20 000) were used to construct null distributions and test the significance of variance components for each hierarchical comparison (Guo & Thompson 1992). Other AMOVA tests were performed with ARLEQUIN 2001 (Schneider *et al*. 2000).

Tajima's Δ statistic (Tajima 1989) and Fu's F_S test (Fu 1997) for selective neutrality were calculated in ARLEQUIN 2001 (Schneider *et al*. 2000). For neutral markers these tests can be used to detect changes in population size. Significant negative Δ and F_S values can be interpreted as signatures of population expansion. We also used mismatch analysis to further explore the demographic evolution of the species. This analysis compares the distribution of the frequency of pairs of individuals who differ by a certain number of nucleotide differences (Slatkin & Hudson 1991; Rogers & Harpending 1992; Schneider & Excoffier 1999). The resulting distribution was tested against the sudden population expansion model as calculated and implemented in ARLEQUIN 2001 (Schneider *et al*. 2000). The test is based on three parameters (Rogers & Harpending 1992): θ_0, θ_1 (θ before and after the population growth), and τ (date of the growth in units of mutational time; $\tau = 2\mu t$, where μ is the mutation rate for the whole sequence and t is the time). The validity of the expansion model was tested by a parametric bootstrap approach, which compares the fit with the expected mismatch distribution of the observed and 100 simulated mismatch distributions. The fit to the expected mismatch distribution was quantified by the sum of squared deviations (SSD) between the observed and simulated distributions on one hand and the expected distribution on the other. This distribution is usually unimodal for lineages that have undergone a recent bottleneck or population expansion and multimodal for a lineage whose population is in demographic equilibrium or is subdivided into several units.

The program MIGRATE, a Monte Carlo Markov Chain method based on the coalescent theory (Beerli & Felsenstein 1999), was used to estimate the products of effective female population size and mutation rate ($N_{e(f)}\,\mu$) and effective female population size and migration rate ($N_{e(f)}\,m$). F_{ST} estimates of effective population sizes and migration rates were used as initial values. Ten short chains with 10 000 sampled genealogies each and three long chains with 100 000 sampled genealogies each were run. One of every 20 reconstructed genealogies was sampled. Heating was set to be active, with four temperatures of 1.0, 1.5, 3.0 and 6.0.

Results

High levels of sequence variation in mackerel

A 414-bp portion of the mitochondrial control region was sequenced for 196 individuals of S*comber scombrus* from three Mediterranean Sea populations (Kavala, Bari, Barcelona) and one from the Atlantic Ocean (Olhão) (Table 1 and Fig. 1). The new sequence data were combined with 272-bp mitochondrial control region sequences of 66 individuals from one Mediterranean Sea (Adriatic) and four Atlantic Ocean (Southern, Western, North Sea, and Canada) populations (Nesbø *et al*. 2000) (Table 1 and Fig. 1). After gap exclusion, the alignment was finally reduced to 272 sites

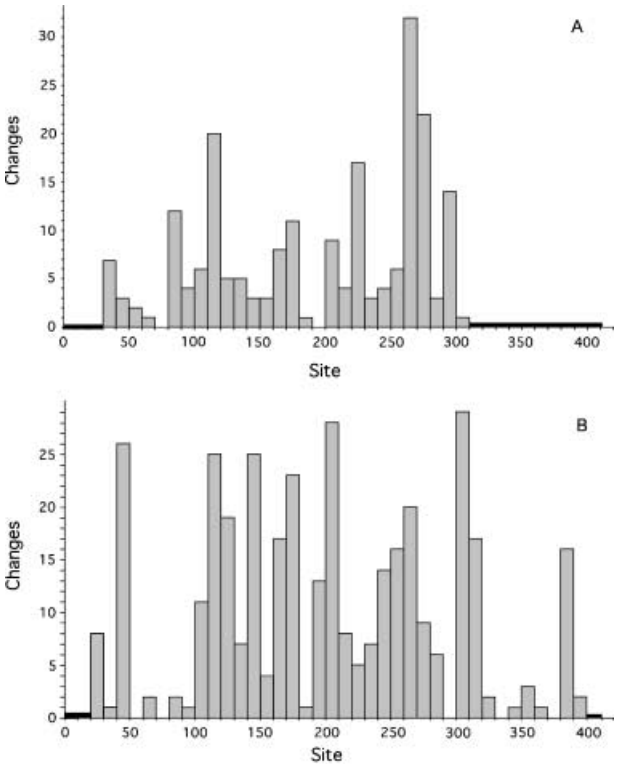

Fig. 2 Distribution of nucleotide substitutions across the studied mitochondrial control region sequences. A — *Scomber scombrus*; B — *Scomber japonicus*. In both cases, position + 1 is designed as the first nucleotide after the PCR primer L-Pro 1. Black boxes indicate regions not included in the final phylogenetic analyses.

because of the shorter length of the sequences reported by Nesbø *et al*. (2000) (Fig. 2A). A total of 102 nucleotide polymorphisms were detected, and 60 were parsimony informative. Indels occurred at 16 sites. A total of 89 transitions and 36 transversions were scored. Nucleotide substitutions were not homogeneously distributed across the sequence, but grouped into two distinct regions, between positions 80–180 and 200–300 (Fig. 2A; position + 1 was defined as the first nucleotide after the L-Pro 1 primer). Most variability was centred at positions 120–130 and 260–280. Variable sites defined a total of 168 different haplotypes. The most abundant haplotype was shared by 37 specimens (10 from Kavala, 12 from Bari, 10 from Barcelona, four from Olhão, and one from the southern Atlantic Ocean) whereas the next most common occurred only in seven individuals. A total of 138 haplotypes were unique. As a result overall haplotype diversity was high (mean value = 0.98 ± 0.01). Population genetic statistics are listed in Table 1.

All mackerel sequences were pooled and departure from neutral expectations tested. Both Tajima's Δ-value (–1.989, $P < 0.001$) and Fu's F_S value (–24.788, $P < 0.01$), based on the total number of segregating sites, were significant, indicating that the number of rare haplotypes is higher than expected under equilibrium.

High levels of sequence variation in chub mackerel

The left domain of the mitochondrial control region was sequenced for 263 specimens of *S. japonicus*. The initial multiple alignment of 414 positions was reduced to 387 sites after gap exclusion. Indels occurred at 10 sites. A total of 104 polymorphic sites were observed, of which 54 were parsimony informative. Nucleotide changes were not randomly distributed across sites (Fig. 2B). Most sites showed high levels of nucleotide substitution whereas positions 50–100, 180–190, 290–300, and 320–380 had little variation (Fig. 2B; position + 1 was defined as the first nucleotide after the L-Pro 1 primer). A total of 84 transitions and 40 transversions were scored. There were 190 different haplotypes and haplotype diversity was 0.99 ± 0.00. The most common haplotype was found in 24 individuals (four from Kavala, one from Lampedusa, six from Messina1, two from Messina2, five from Barcelona, and six from Olhão) and there were 168 unique haplotypes. A summary of polymorphism parameters is given in Table 1.

Neutrality tests were performed by pooling all chub mackerel sequences and estimating the Tajima's Δ statistic and Fu's *F*s value. Significant departures from equilibrium were found ($D = -1.822$; $F_S = -24.569$; $P < 0.001$).

Phylogenetic relationships

The NJ phylogenies (Tamura-Nei with gamma correction) based on mackerel and chub mackerel mitochondrial control region haplotypes are shown in Figs 3 and 4, respectively. Both topologies lacked bootstrap support at internal nodes. Even though samples were collected during the spawning season of each species, in general there was no apparent relationship between haplotypes from the same spawning ground. Haplotype networks for both species were also reconstructed based on reduced data sets (only transversions) with parsimony analyses (insets Figs 2 and 3). As in the case of the NJ trees, networks showed low phylogeographic structure.

Differential population genetic structuring of scomber species

Φ_{ST} population pairwise comparisons within each species of Scomber exhibit no clear differentiation (Tables 2 and 3). Of the 36 possible Φ_{ST} comparisons within *S. scombrus* only three showed significant statistical values, all involving Bari (Olhão, Adriatic and Canada), whereas none of the pairwise Φ_{ST} comparisons was significant within *S. japonicus*.

The differential population structuring of the analysed mackerel and chub mackerel populations was further

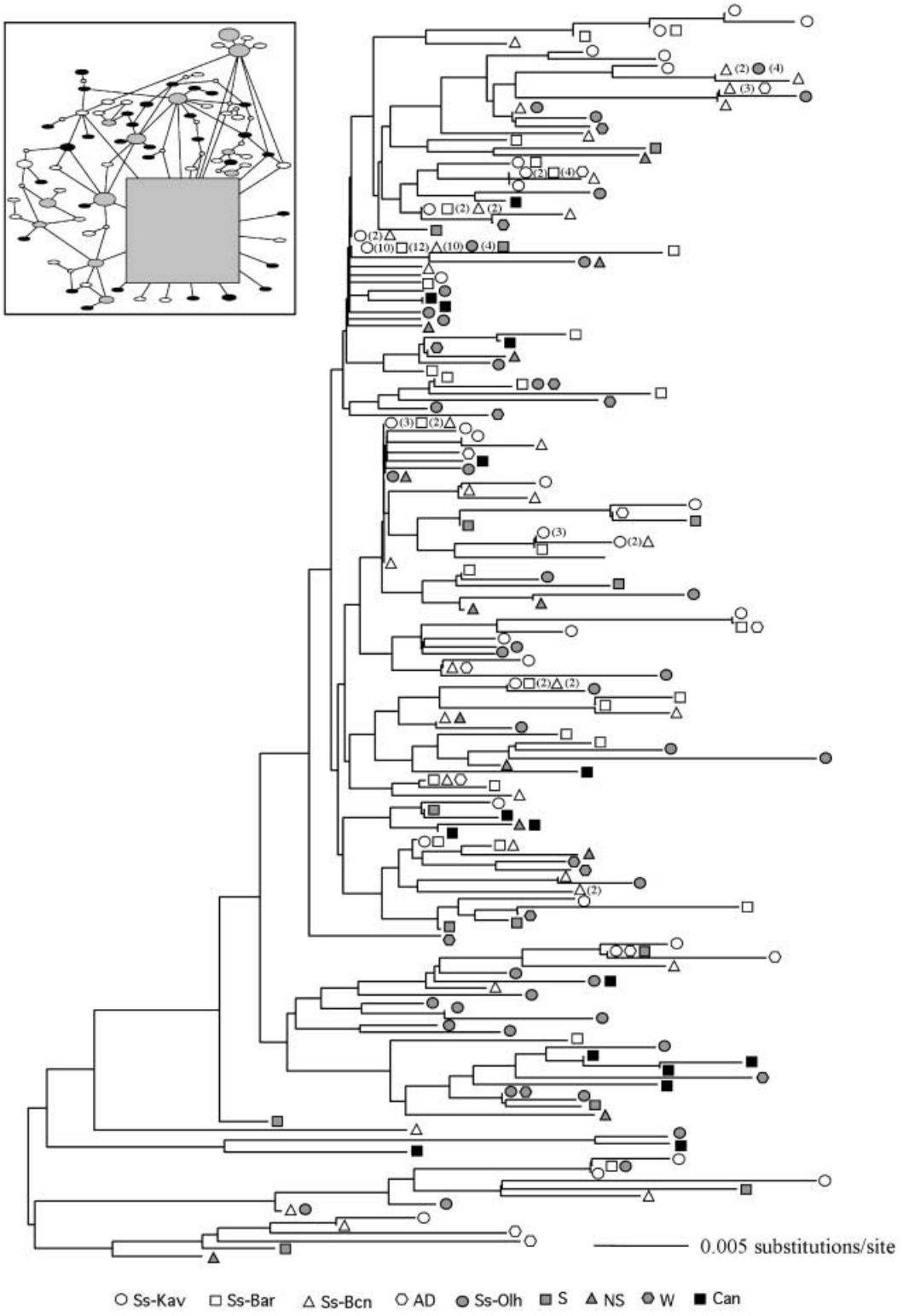

Fig. 3 Neighbour-joining tree constructed using Tamura and Nei distances with a gamma shape parameter of 0.68 for *Scomber scombrus*. Inset: Network of mtDNA control region haplotypes (only transversions). Genealogical relationships were estimated by the parsimony method of Templeton *et al.* (1992). White symbols represent Mediterranean haplotypes; black symbols are the Atlantic haplotypes; grey symbols share Mediterranean and Atlantic haplotypes. The size of ovals is proportional to the number of individuals sharing a particular haplotype. Each single line indicates one mutation between haplotypes (small circles dividing single lines represent missing haplotypes). The haplotype marked as a rectangle had the highest outgroup probability.

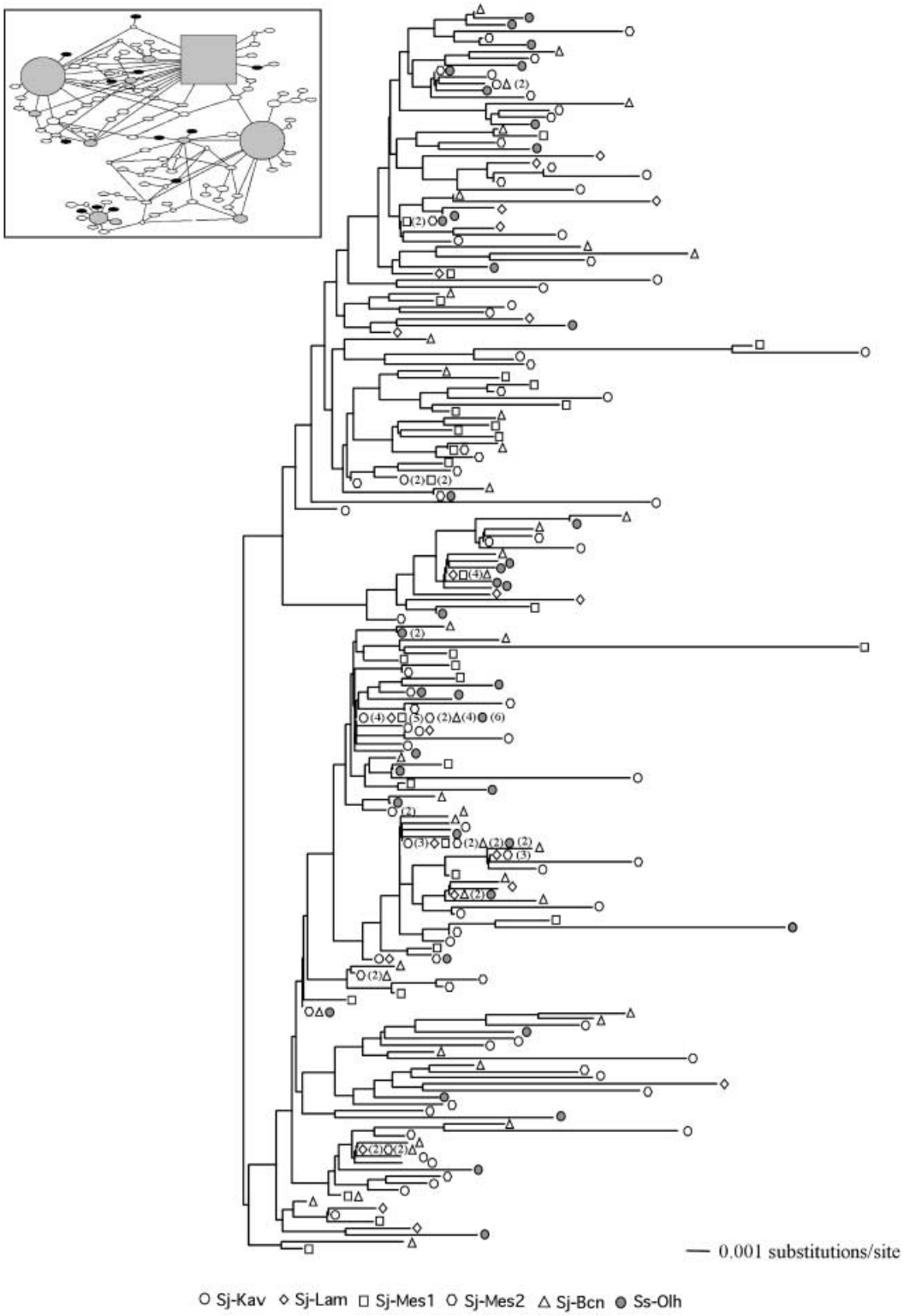

Fig. 4 Neighbour-joining tree constructed using Tamura and Nei distances with a gamma shape parameter of 0.45 for *Scomber japonicus*. Inset: Network of mtDNA control region haplotypes (only transversions). Genealogical relationships were estimated by the parsimony method of Templeton *et al*. (1992). White symbols represent Mediterranean haplotypes; black symbols are the Atlantic haplotypes; grey symbols share Mediterranean and Atlantic haplotypes. The size of ovals is proportional to the number of individuals sharing a particular haplotype. Each single line indicates one mutation between haplotypes (small circles dividing single lines represent missing haplotypes). The haplotype marked as a rectangle had the highest outgroup probability.

Table 2 Pairwise Φ_{ST} (below diagonal) and associated *P*-values (above diagonal) among *S. scombrus* samples

	Ss-Kav	Ss-Bar	Ss-Bcn	Ss-Olh	AD	S	W	NS	Can
Ss-Kav	—	0.067	0.050	0.002	0.057	0.765	0.025	0.255	0.002
Ss-Bar	0.011	—	0.012	0.000	0.000	0.016	0.116	0.079	0.000
Ss-Bcn	0.011	0.022	—	0.255	0.036	0.209	0.072	0.267	0.005
Ss-Olh	0.026	0.047*	0.003	—	0.085	0.661	0.096	0.462	0.078
AD	0.034	0.117*	0.044	0.025	—	0.884	0.003	0.094	0.074
S	−0.017	0.044	0.009	−0.009	−0.033	—	0.086	0.723	0.207
W	0.047	0.026	0.037	0.028	0.088	0.038	—	0.110	0.149
NS	0.004	0.021	0.004	−0.003	0.035	−0.018	0.028	—	0.188
Can	0.06	0.081*	0.047	0.017	0.042	0.015	0.021	0.015	—

*significant at $P < 0.001$ (after Bonferroni correction).

Table 3 Pairwise Φ_{ST} (below diagonal) and associated *P*-values (above diagonal) among *S. japonicus* samples

Sj-Kav	Sj-Mes1	Sj-Mes2	Sj-lam	Sj-Bcn	Sj-Olh	
Sj-Kav	—	0.018	0.227	0.773	0.735	0.544
Sj-Mes1	0.023	—	0.132	0.082	0.043	0.073
Sj-Mes2	0.004	0.099	—	0.611	0.706	0.490
Sj-Lam	−0.008	0.019	−0.005	—	0.977	0.707
Sj-Bcn	−0.005	0.018	−0.005	−0.015	—	0.863
Sj-Olh	−0.002	0.016	−0.002	−0.007	−0.008	—

No significant values after Bonferroni correction.

explored by hierarchical AMOVA tests (Table 4). Significant levels of genetic structuring were found among *S. scombrus* ($\Phi_{ST} = 0.024$; $P = 0.0001$) but not among *S. japonicus* populations ($\Phi_{ST} = 0.003$; $P = 0.24$). When analyses were computed including samples only from locations (Kavala, Barcelona and Olhão) common to both species, the result *for S. scombrus* was still significant ($\Phi_{ST} = 0.014$; $P = 0.05$). Additional AMOVA tests were performed by pooling populations into different alternative structures by maximizing Φ_{CT} values for *S. scombrus* (Table 4). The maximum Φ_{CT} value for two, three, four- and five-gene pools (see Material and Methods)

Table 4 Genetic structuring of Scomber populations

		Observed partition		
Stucture tested	Variance	% total	Φ statistics	*P*
S. japonicus				
1. One gene pool (Sj-Kav, Sj-Lam, Sj-Mes1, Sj-Mes2, Sj-Bcn, Sj-Olh)				
Among populations	0.009	0.26	$\Phi_{ST} = 0.003$	0.237
Within populations	3.622	99.74		
S. scombrus				
1. One gene pool (Ss-Kav, Ss-Bar, Ss-Bcn, Ss-Olh, AD, S, W, NS, Can)				
Among populations	0.077	2.41	$\Phi_{ST} = 0.024$	0.0001
Within populations	3.134	97.59		
2. Two gene pools* (Ss-Kav, Sj-Bar, Ss-Bcn, S, N, Ss-Olh, W, Can) (AD)				
Among groups	0.112	3.56	$\Phi_{CT} = 0.036$	0.122
Among populations/group	0.063	1.99	$\Phi_{SC} = 0.021$	< 0.001
Within populations	2.972	94.45	$\Phi_{ST} = 0.056$	< 0.001
3. Three gene pools* (Ss-Kav, Sj-Bar, Ss-Bcn, S, N, Ss-Olh, W) (Can) (AD)				
Among groups	0.111	3.55	$\Phi_{CT} = 0.036$	0.019
Among populations/group	0.048	1.55	$\Phi_{SC} = 0.016$	< 0.001
Within populations	2.972	94.9	$\Phi_{ST} = 0.051$	< 0.001
4. Four gene pools* (Ss-Kav, Sj-Bar, Ss-Bcn, S, N, Ss-Olh) (W) (Can) (AD)				
Among groups	0.099	3.17	$\Phi_{CT} = 0.032$	0.017
Among populations/group	0.042	1.36	$\Phi_{SC} = 0.014$	0.002
Within populations	2.972	95.48	$\Phi_{ST} = 0.045$	< 0.001
5. Five gene pools* (Ss-Kav, Sj-Bar) (Ss-Bcn, S, N, Ss-Olh) (W) (Can) (AD)				
Among groups	0.069	2.31	$\Phi_{CT} = 0.023$	0.003
Among populations/group	0.016	0.53	$\Phi_{SC} = 0.005$	< 0.001
Within populations	2.908	97.16	$\Phi_{ST} = 0.028$	< 0.001

*by maximizing Φ_{CT}.

corresponded successively to Adriatic, Canada, western Atlantic Ocean, and eastern Mediterranean Sea (Bari + Kavala) vs. all other populations (Table 4). All of these gene pool comparisons were significant ($P < 0.05$) except for the two-gene pool structure. The same results (not shown) were obtained when the Adriatic sample, which is the smallest in size and may include nonspawning individuals (Nesbø *et al*. 2000), was excluded from the AMOVA analyses. A putative Atlantic Ocean-Mediterranean Sea two gene-pool population structuring was tested and resulted in a extremely low Φ_{CT} (0.008) non-significant value (not shown). Interestingly, although most of the observed variation could be ascribed to differences within populations (Φ_{ST}), significant amounts of the variance could also be attributed to differences among populations within a group (Φ_{SC}) for all alternative hypotheses tested ($P < 0.05$) (Table 4).

Patterns of historical demography

In order to recover the details of historical population expansion, we applied a simplified model of expansion to our data. This allowed us to estimate effective female population size and the time and rate of expansion. The mismatch distribution of mackerel was distinctively unimodal (Fig. 5A) and the expansion model was not rejected ($P_{SSD} = 0.84$). The parameters of the expansion model were as follows: $\theta_0 = 2.676$, $\theta_1 = 60.654$ and $\tau = 3.556$. The chub mackerel also showed a unimodal shape (Fig. 5B), fitting the theoretically expected curve ($P_{SSD} = 0.59$) under the sudden expansion model. The parameters of the expansion model were as follows: $\theta_0 = 0.011$, $\theta_1 = 35.763$ and $\tau = 7.554$. Estimated effective female population size after expansion (θ_1) was 30 and 3000 times higher than before expansion (θ_0) for *S. scombrus* and *S. japonicus*, respectively.

Gene flow and effective female population sizes were also estimated by coalescence methods. Extreme asymmetry of migrant exchange was detected in both species

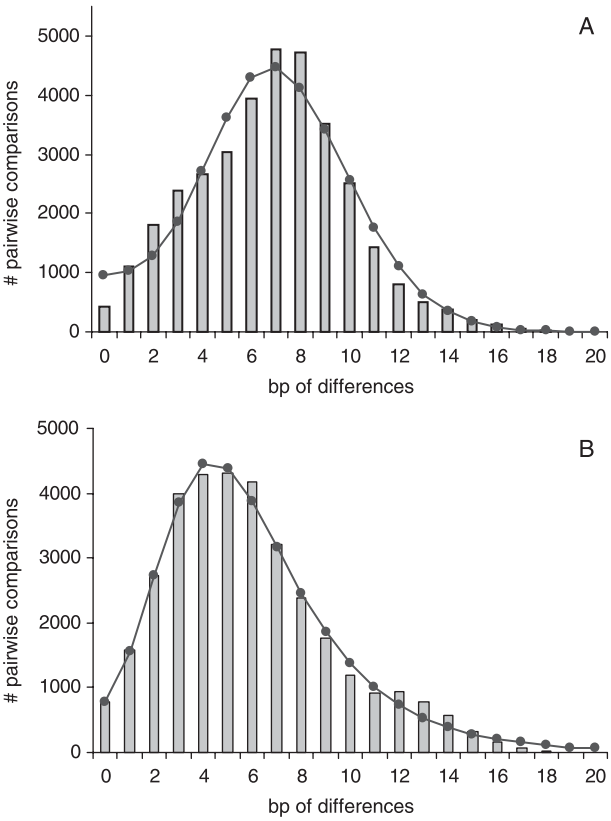

Fig. 5 Mismatch distributions for all pairwise combinations of: A–262 individuals for *Scomber scombrus* ($P_{SSD} = 0.87$); B–263 individuals for *Scomber japonicus* ($P_{SSD} = 0.59$). Observed distribution (bars) conformed to the expected Poisson distribution (line) under a model of sudden population expansion as implemented in ARLEQUIN 2.001.

(Tables 5 and 6). According to the results, mackerels migrate from all populations towards Bari (for instance, this population receives $4Nm = 588$ from Olhão, 245 from Barcelona and 211 from Kavala) whereas chub mackerels

Table 5 Gene-flow estimates for *S. scombrus*

			4*Nm*†								
Population	Theta [2*Ne* μ]	*Ne**	1	2	3	4	5	6	7	8	9
1 Ss-Kav	0.010	4.4×10^4		10	11	5	1	4	2	1	6
2 Ss-Bar	0.051	2.3×10^5	211		245	588	9	14	69	14	16
3 Ss-Bcn	0.044	2×10^5	5	5		23	15	66	15	73	0
4 Ss-Olh	0.102	4.6×10^5	32	26	4		12	38	0	0	50
5 AD	0.128	5.8×10^5	27	0	5	77		14	12	7	4
6 S	0.071	3.2×10^5	8	18	2	25	37		22	60	33
7 W	0.134	6.1×10^5	7	32	0	52	10	47		4	4
8 NS	0.039	1.8×10^5	0	0	1	33	0	13	10		31
9 Can	0.041	1.8×10^5	10	0	2	102	47	4	0	16	

All values within bounds of 95% confidence limit.
*Using a $\mu = 1.1 \times 10^{-7}$ per site per year (McMillan & Palumbi 1997).
†Columns are donor populations whereas rows are receiving populations.

Table 6 Gene-flow estimates for *S. japonicus*

Population	Theta [2*Ne* μ]	*Ne**	4*Nm*† 1	2	3	4	5	6
1 Sj-Kav	1.357	6.2×10^6		1138	304	233	170	38
2 Sj-Lam	0.054	2.4×10^5	1		9	11	5	< 1
3 Sj-Mes1	0.030	1.4×10^5	4	3		10	2	7
4 Sj-Mes2	0.101	4.6×10^5	29	38	96		36	64
5 Sj-Bcn	0.140	6.4×10^5	92	< 1	133	204		375
6 Sj-Olh	0.064	2.9×10^5	102	81	6	37	71	

All values within bounds of 95% confidence limit.
*Using a $\mu = 1.1 \times 10^{-7}$ per site per year (McMillan & Palumbi 1997).
†Columns are donor populations whereas rows are receiving populations.

migrate from all populations mostly to Kavala and secondarily to Barcelona. There are however, important differences in the absolute number of migrants between the two species, as overall the chub mackerel exchanges individuals five times more within the Mediterranean Sea than the mackerel (this result may only indicate the panmictic nature of *S. japonicus* and should be taken with caution). Interestingly, within the Mediterranean Sea, Adriatic and Kavala showed the largest effective female population size for *S. scombrus* and *S. japonicus*, respectively (as inferred from Tables 5 and 6, column $2N_{e(f)}$ μ). If a mutation rate for the control region of teleosts is assumed to be 1.1×10^{-7} per site per year (McMillan & Palumbi 1997; Bargelloni *et al*. 2003), the effective female population sizes ($N_{e(f)}$) range between 4.4×10^4 and 6×10^5 and between 1.4×10^5 and 6×10^6 for mackerel and chub makerel, respectively.

Discussion

This study provides a thorough genetic analysis on the Mediterranean Sea populations of mackerel and chub mackerel. It is the first work determining population genetic structure of chub mackerel and expands previous work by Nesbø *et al*. (2000) on Atlantic Ocean populations of mackerel. The nucleotide sequence of the 5′-end of the mitochondrial control region was determined in an average of 46 spawning individuals per population per species from the eastern, central, and western Mediterranean Sea, as well as the southern Iberian Atlantic Ocean waters. The new mitochondrial control region sequence data were compared with the corresponding sequences previously determined by Nesbø *et al*. (2000) from four Atlantic Ocean (Southern, Western, North Sea, Canada) and one Mediterranean Sea (Adriatic Sea) mackerel populations. The mitochondrial control region sequences showed adequate levels of polymorphism for the question at hand and revealed high levels of haplotypic diversity that were similar to those found in the Atlantic Ocean mackerel (Nesbø *et al*. 2000) and slightly higher than those of other marine fishes (Rocha-Olivares *et al*. 1999; Beheregaray & Sunnucks 2001).

Marine migratory fishes with high dispersal capabilities and large effective population sizes are anticipated to show high levels of gene flow and a low degree of differentiation (Nesbø *et al*. 2000; Beheregaray & Sunnucks 2001). According to our results, chub mackerel conforms to this pattern whereas mackerel shows differentiation along an east–west axis over its distribution. The differential structuring of the populations of these two closely related species is supported both by the Φ_{ST} pairwise comparisons and the AMOVA tests. None of the Φ_{ST} estimates is significant for *Scomber japonicus* whereas three pairwise comparisons of *S. scombrus* involving Bari (with Canada, Olhão and Adriatic, respectively) showed evidence of genetic differentiation. The AMOVA tests showed significant levels of genetic structuring among *S. scombrus* but not *S. japonicus* populations. Overall, our analyses indicate that the chub mackerel is panmictic in the Mediterranean Sea and adjacent Atlantic Ocean waters. In contrast, mackerel populations show statistically significant levels of genetic structuring. Mackerel populations are differentiated along an east–west axis: Kavala and Bari vs. Barcelona, Olhão, Southern and Northern vs. Western vs. Canada. Our results on *S. scombrus* agree with previous findings of genetic structuring in the Atlantic Ocean based on mitochondrial control region and cytochrome *b* gene sequence data (Nesbø *et al*. 2000). Even though Nesbø *et al*. (2000) failed to detect significant differentiation between Adriatic and Atlantic Ocean populations based on Φ_{ST} estimates (but see below), a nested clade analysis based on cytochrome *b* haplotypes showed restriction of gene flow between the eastern and western Atlantic Ocean and the Mediterranean Sea (Nesbø *et al*. 2000). Additionally, it is interesting to note that our results on mackerel support a genetic discontinuity for this

species at the Siculo-Tunisian strait (Barhi-Sfar *et al*. 2000) but not at the Gibraltar strait (Bargelloni *et al*. 2003).

Our results on *S. japonicus* are consistent with its cosmopolitan nature and with previous studies on the global phylogeography of the species (Scoles *et al*. 1998). Restriction site analysis of the whole mitochondrial genome and sequence analysis of the mitochondrial cytochrome *b* gene resulted in no significant differentiation between populations from the eastern Mediterranean Sea (Israel coast), Ivory Coast, and South Africa (Scoles *et al*. 1998). Interestingly, an allozyme based study was able to find differences between Mediterranean Sea and Southeastern Atlantic Ocean (off the coast of Argentina) populations of *S. japonicus* (Roldan *et al*. 2000). However, it should be noted that in both of these studies, the samples representing the Mediterranean Sea were from a single location, the number of samples per population was relatively low, and no distinction was made between spawning and nonspawning individuals.

Which of the competing hypotheses that explain the maintenance of marine fish population genetic structure may better explain the differential structuring pattern of chub mackerels and mackerels? Our results show that all significant pairwise comparisons involved the mackerels from Bari, in the southern Adriatic (with the comparison with Ancona in central Adriatic being the most distinct). Moreover, Bari seemed to receive migrants from all other populations and to barely donate any. The peculiarity of Bari may be explained by environmental factors (i.e. hypothesis 1), in particular, the hydrography and circulation patterns of the Adriatic Sea. Two strong gyres separate southern Adriatic from northern and central Adriatic (Ancona samples from Nesbø *et al*. 2000) as well as from the rest of the Mediterranean Sea (Poulain & Cushman-Roissin 2000; Poulain 2001). A genetic discontinuity between central and southern Adriatic was also observed in another small pelagic fish, the anchovy *Engraulis encrasicolus* (Bembo *et al*. 1996). In any case, it is important to note that the sample from central Adriatic (Ancona) was the smallest in size of all, and consisted of non-spawning juveniles (Nesbø *et al*. 2000). This may indicate that early separation of Ancona in the two-gene pool AMOVA test could be spurious, as well as the results of pairwise comparisons involving this sample.

A hypothetical lower dispersal capability of mackerels could affect gene flow among populations over long distances and be responsible for their emerging phylogeographic structure (hypothesis 2). This may explain the early separation of the mackerel sample from Canada in the AMOVA tests, but not the differential mackerel population structuring in the Mediterranean Sea. Physiological studies on muscle fibre activity show that mackerels are in fact more powerful swimmers than chub mackerels. Mackerels are obligate swimmers due to the absence of a swimbladder and the necessity for gill ventilation. They show variation of muscle kinetics along the body resulting in a fast and strong swimming style (Wardle & Videler 1993). In contrast, chub mackerels have a swimbladder that enables the fish to hold position off the bottom of the sea. They display homogeneous muscle activity at all body positions, which allows them to display wider swimming styles (Shadwick *et al*. 1998).

An alternative explanation for the phylogenetic patterns may lie with life-history factors (hypothesis 3). Population structure may be created by homing of adults to specific spawning grounds and be enhanced by larval retention in a particular site. Homing of adults has been demonstrated for mackerel, e.g. in the western Atlantic Ocean (Studholme *et al*. 1999), and has been suggested as the mechanism underlying population structuring in Atlantic Ocean mackerel (Nesbø *et al*. 2000). In contrast, homing is not well known for the chub mackerel. The recruitment of the chub mackerel is clearly different from that of the mackerel and other scombrids. The larvae of the chub mackerel exhibit a voracious behaviour with high daily rations (87% of body weight) (Hunter & Kimbrell 1980) when compared with the mackerel (25–50% of body weight) (Peterson & Ausubel 1984). Additionally, the fecundity of the chub mackerel (one million eggs per year) is more than double that of the mackerel (400 000 eggs per year) (Metz & Myers 1996). Hence, it is possible that the larger fecundity and the more voracious behaviour of the chub mackerel may act against the local recruitment of this species and prevent genetic structuring of its populations.

Marine environments are often seen as open habitats in which isolation by distance is the main mechanism that may promote speciation (Palumbi 1994). However, several studies have demonstrated the existence of marine physical barriers (e.g. strong ocean currents, gyres, etc.) that trigger intraspecific genetic fragmentation in different species. For instance, there are phylogeographic breaks for sea bass around the Siculo-Tunisian strait (Bahri-Sfar *et al*. 2000), for anchovies in the Adriatic Sea (Bembo *et al*. 1996), for some sparid species around the Gibraltar strait (Bargelloni *et al*. 2003), for gobies in the Florida keys (R̨ber *et al*. 2003), and for bigeye tuna around the Cape of Good Hope (Chow *et al*. 2000). Interestingly, however, the same geographical regions do not necessarily restrict gene flow of other related species of marine pelagic fishes (Bargelloni *et al*. 2003; Bernardi *et al*. 2003). Overall, our results on Scomber suggest that life-history traits such as homing behaviour or larval retention (Jones *et al*. 1999; Knutsen *et al*. 2003) may be important in determining the sensitivity of closely related fish species to physical barriers in the marine realm. Further studies on the biology of marine pelagic and demersal fish species as well as refined population genetic studies concentrated over short geographical ranges around transition zones are needed to better understand the observed differences (Bargelloni *et al*. 2003).

Alternatively, the differences observed between the two Scomber species may be due to historical factors such as a more recent establishment of chub mackerel populations

in the Mediterranean Sea, to effects of genetic drift, or to selection. In this regard, it is interesting to note that in both species, the neutrality test rejected equilibrium expectations. Departures of the mackerel and chub mackerel Mediterranean Sea populations from the levels of genetic variation expected may occur if these populations experienced rapid expansion in the past, as the mismatch results seem to point out. However, current expansion models and statistics seem unable to capture recent, human-induced contractions and expansions of population sizes which, in the case of exploited species, may be much more relevant to persistence of species than historic natural changes.

The results have important implications for fisheries management of both species in the Mediterranean Sea. The lack of structure found in *S. japonicus* in the Mediterranean Sea is consistent with a 'one stock' management policy. In contrast, the finding that *S. scombrus* populations are structured in the Mediterranean Sea and that their migration patterns are asymmetric towards the Southern Adriatic, point out the need to take into account recruitment along an east–west axis to avoid local over-exploitation and decline, and to ensure effective sustainability of each of the putative Mediterranean Sea stocks (as already suggested by Nesbø *et al*. 2000 for the Atlantic Ocean mackerel stocks). In particular, the eastern Mediterranean Sea populations seem to be more distinct (there is apparent gene flow between western Mediterranean Sea and Atlantic Ocean mackerel populations) but also more sensitive to human pressure: mackerel is now considered extinct in the northern and western part of the Black Sea, while its effective number has considerably decreased in the Sea of Marmara (Prodanov *et al*. 1996). Hence, conservation actions should preferentially concentrate on this stock.

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Rafael Zardoya and C. Grande are interested in understanding the evolutionary mechanisms that are involved in the generation of biodiversity. Their research interests include molecular systematics and population genetics mainly in fish, amphibians, and gastropods. Rita Castilho heads the Biodiversity and Conservation Unit (BioCon) at CCMAR focusing her research on a variety of evolutionary genetic problems including island biogeography, phylogeography and population genetics of gastropods, amphibians and fish. S. Caetano was a lab technician at BioCon and is presently starting her PhD in Switzerland. Presently, the main research interests of Grigorios Krey and L. Favre-Krey include lipid metabolism and nutrition of fish and genetic cataloguing of European marine fish species. Tomaso Patarnello and S. Marcato work on population genetics of marine organisms (particularly fish). Their interest is mostly focused on the investigation of microevolutionary processes leading to population isolation in the marine environment.
