African Journal of Marine Science
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t911470580

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Online publication date: 10 June 2011

To cite this Article Kaouèche, M. , Bahri-Sfar, L. , González-Wangüemert, M. , Pérez-Ruzafa, Á and Ben Hassine, OK(2011) 'Allozyme and mtDNA variation of white seabream *Diplodus sargus* populations in a transition area between western and eastern Mediterranean basins (Siculo-Tunisian Strait)', African Journal of Marine Science, 33: 1, 79 — 90
To link to this Article: DOI: 10.2989/1814232X.2011.572342
URL: http://dx.doi.org/10.2989/1814232X.2011.572342

African Journal of Marine Science 2011, 33(1): 79–90
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AFRICAN JOURNAL OF MARINE SCIENCE
ISSN 1814–232X EISSN 1814–2338
doi: 10.2989/1814232X.2011.572342

Allozyme and mtDNA variation of white seabream *Diplodus sargus* populations in a transition area between western and eastern Mediterranean basins (Siculo-Tunisian Strait)

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Manuscript received May 2010; accepted January 2011

To investigate the possible influence of the Siculo-Tunisian Strait on the genetic structure of white seabream *Diplodus sargus*, 13 polymorphic allozyme loci and a fragment of the cytochrome *b* mitochondrial DNA were analysed. Allozyme data indicated a moderate but significant differentiation between some north-eastern (Bizerta, Ghar El Melh Lagoon and Mahdia) and southern (Gabes Gulf and El Biban Lagoon) samples. This heterogeneity was also highlighted after removing *PGM and *PGI-1** loci which may be under selection. These results can be explained by the chaotic genetic patchiness hypothesis. In contrast, the mtDNA data indicated genetic homogeneity among localities showing the absence of structure in white seabream populations across the Siculo-Tunisian Strait. Historical demography of this species suggests that it has undergone a recent population expansion as a consequence of a bottleneck event during the Pleistocene glaciations.**

Keywords: chaotic genetic patchiness, cytochrome *b*, discrepancy, genetic connectivity, Tunisian coasts, Wahlund effect

Introduction

The Mediterranean Sea is characterised by a complex circulation where two main water bodies meet (Modified Atlantic Water and Levantine Intermediate Water; Astraldi et al. 1999). The geography is also influenced by the presence of physical barriers such as straits and channels (Béranger et al. 2004). The most important Mediterranean barriers are the Gibraltar Strait, Almería-Orán oceanographic front, Siculo-Tunisian Strait, and the hydrographical isolation of the Aegean-Ionian and Adriatic Seas (Astraldi et al. 1999, Patarnello et al. 2007, Pérez-Losada et al. 2007).

For some species, these areas are considered as gene-flow barriers, regardless of their dispersal ability (Borsa et al. 1997, Arculeo et al. 2003, Bargelloni et al. 2003, Patarnello et al. 2007), leading to the genetic differentiation among populations.

The Siculo-Tunisian Strait, located between Cap Bon in Tunisia and Mazara del Vallo in Italy, provides the direct interface between the eastern and western Mediterranean basins (Quignard 1978). Along this transition area, two waterbodies circulate with different hydrological, physical and chemical characteristics. This area has been described as a transition zone that can bring about population genetic differentiation (Bahri-Sfar et al. 2000, Stefanni and Thorley 2003, Zardoya et al. 2004, Mejri et al. 2009, Zitari-Chatti et al. 2009). Research on the patterns of marine population structure in this area, using different molecular markers, can contribute to a better understanding of the role of this complex circulation on disrupting gene flow and what the consequences of this may be.

The Sparidae, commonly named seabreams, are highly diversified demersal fish found at variable depths (0–250 m) in temperate and tropical marine waters (Bauchot and Hureau 1986). The white seabream *Diplodus sargus* (Linnaeus 1758) is one of the most important commercial sparids found throughout the Mediterranean Sea (Fischer et al. 1987). They live in coastal rocky reef areas and coastal lagoons and spawn in the open sea from March to June (Leboulleux 1992). The larvae dispersion duration in the sea is between three to four weeks until arriving at their recruitment sites (Vigliola 1998).

Because of its ecological and economic importance, several studies, using different molecular markers, have been conducted to compare white seabream population genetic structure in the Atlanto–Mediterranean region. No

appreciable genetic differences were detected between Atlantic and Mediterranean *D. sargus* populations using several markers: allozymes, mitochondrial control region and cytochrome *b*, the first intron of the S7 ribosomal protein gene, and nine microsatellite loci (Bargelloni et al. 2005, Domingues et al. 2007, González-Wangüemert et al. 2010, 2011). Despite a lack of global structure, the Azores population showed high and significant genetic differentiation with all other samples (González-Wangüemert et al. 2010, 2011). The authors attributed this differentiation to the hydrodynamic and historical factors acting as barriers to the free dispersal of white seabream in this region.

Among Mediterranean populations, allozyme studies have also shown significant genetic differences at temporal and spatial scales among several localities of the western Mediterranean Sea (Lenfant and Planes 1996, 2002, Planes and Lenfant 2002, González-Wangüemert et al. 2004, 2007). Some studies showed genetic differences at small spatial scales, mainly among island and coastal populations (Lenfant and Planes 1996, 2002, Planes and Lenfant 2002), and others reported significant differences between populations situated <20 km apart that maintained lower genetic fluxes between them than with other localities located hundreds of kilometres away (González-Wangüemert et al. 2004, 2007). Local oceanographic features and heterogeneity on a microgeographical scale or 'chaotic genetic patchiness', considered as the non-random mixing of larvae between cohorts (Johnson and Black 1984), are the two hypotheses advanced to explain such variations (Lenfant and Planes 1996, González-Wangüemert et al. 2004). Temporal variations in genetic signatures, resulting from the mixing of differentiated larval pools, were also highlighted among *D. sargus* cohorts. Differentiation among cohorts was assigned to selection processes and/or large variation in the reproductive success of individuals (genetic drift) (Lenfant and Planes 2002, Planes and Lenfant 2002, González-Wangüemert et al. 2007).

Recently, mitochondrial DNA data (cytochrome *b*, control region) and nine microsatellite loci have detected moderate genetic differentiation between some western Mediterranean samples and Bizerta locality (Tunisia) near the Siculo-Tunisian Strait (González-Wangüemert et al. 2010, 2011). Despite the fact that the genetic structure of western Mediterranean white seabream is well documented, populations from the eastern Mediterranean Sea, and especially in the transition area between the two basins (Tunisian coasts), have not been studied enough and on account of insufficient sampling have provided only partial results.

The use of several genetic markers can be of great interest because each marker reflects independent evolutionary histories in the gene tree, which enables a deeper understanding of marine population structure (Sala-Bozano et al. 2009). Although allozymes reveal lower variability than mitochondrial DNA analysis, they have successfully been used to describe population structure of different marine species (Pérez-Losada et al. 1999, Arculeo et al. 2003, Lo Brutto et al. 2004). Maternally inherited mitochondrial DNA shows relatively faster evolutionary changes and seems to be more helpful in detecting recent evolutionary events, particularly for species with high levels of gene flow such as marine pelagic fish (Hauser and Ward 1998, Avise 2005).

The aim of this study was to investigate the possible influence of the Siculo-Tunisian Strait on gene flow and, thus, on the genetic structure of white seabream populations, using two distinct genetic markers (allozymes and mitochondrial DNA). Historical demography of populations in this transitional area is also investigated.

Materials and methods

Sampling

A total of 164 white seabream, with total lengths ranging between 8.5 and 30.7 cm, was collected from the north-eastern and southern Tunisian coast (Lybico-Tunisian Gulf). To check for most of the environmental variability on both sides of the Siculo-Tunisian Strait boundary, five samples were collected from two coastal lagoons (Ghar El Melh in the north and El Biban in the south) and three marine localities (Bizerta at the north and Mahdia and Gabes Gulf at the south) (Figure 1). For each specimen, liver and muscle tissues were removed and kept at −20 °C for enzymatic analyses. Tissues for mtDNA analyses were placed in 95% ethanol until DNA extraction.

Allozyme electrophoresis

All individuals were used in allozyme electrophoresis. Each piece of muscle was homogenised in an equal volume of Tris buffer (pH = 6.8) and centrifuged at 13 000 rpm for 30 min at 4 °C. For liver tissue, an additional half volume of toluene was added to the extraction buffer. The supernatant was stored at −20 °C. Electrophoresis was performed on starch gel using two buffers (Tris-citrate 8 [TC8] and Tris-citrate 6.7 [TC 6.7]) according to Pasteur et al. (1987). Nine enzymatic systems, showing clear zymograms, were used for analysis: aspartate aminotransferase EC 2.6.1.1 (AAT; TC 6.7), alcohol deshydrogenase EC 1.1.1.1 (ADH; TC 8), esterase EC 3.1.1 (EST; TC 6.7), isocitrate deshydrogenase EC 1.1.1.4.2 (ICD; TC 8), lactate deshydrogenase EC 1.1.1.27 (LDH; TC 8), malate deshydrogenase EC 1.1.1.37 (MDH; TC 6.7), glucose phosphomutase EC 5.4.2.1 (PGM; TC 8), glucose phosphate isomerase EC 5.3.1.9 (PGI; TC8) and superoxydase dismutase EC 1.15.1.1 (SOD; TC 8) (Tab.2). Thirteen polymorphic loci were scored (*AAT-1**, *AAT-2**, *EST-1**, *EST-2**, *EST-3**, *ICD-1m**, *ICD-1l**, *ICD-2**, *MDH-1**, *PGM**, *PGI-1**, *PGI-2** and *SOD**). Nomenclature of loci and alleles was written according to Shaklee (1990).

DNA extraction

A total of 66 individuals from the five collection sites were included in the DNA analyses. Total genomic DNA was extracted from small (3–5 mg) sections of tissue following Sambrook et al. (1989). The extracted DNA was re-suspended in elution buffer and stored at −20 °C until further use. A 671-bp fragment of the cytochrome *b* region (mtDNA) was amplified using the universal primers ctbd2 (5'AATGTGAAAAACCACCGTTG3') and cbtr2 (5'CGGTTACAAGRCCG3') (Jousson et al. 2000). Reactions of 25 µl total volume containing 2.5 µl of 10× buffer (Ecogen), 1.5 mM $MgCl_2$ (Ecogen), 200 µM dNTP mix, 0.5 U Taq DNA polymerase (Ecogen), 0.25 µM each primer and 1 µl of DNA (5–50 ng).

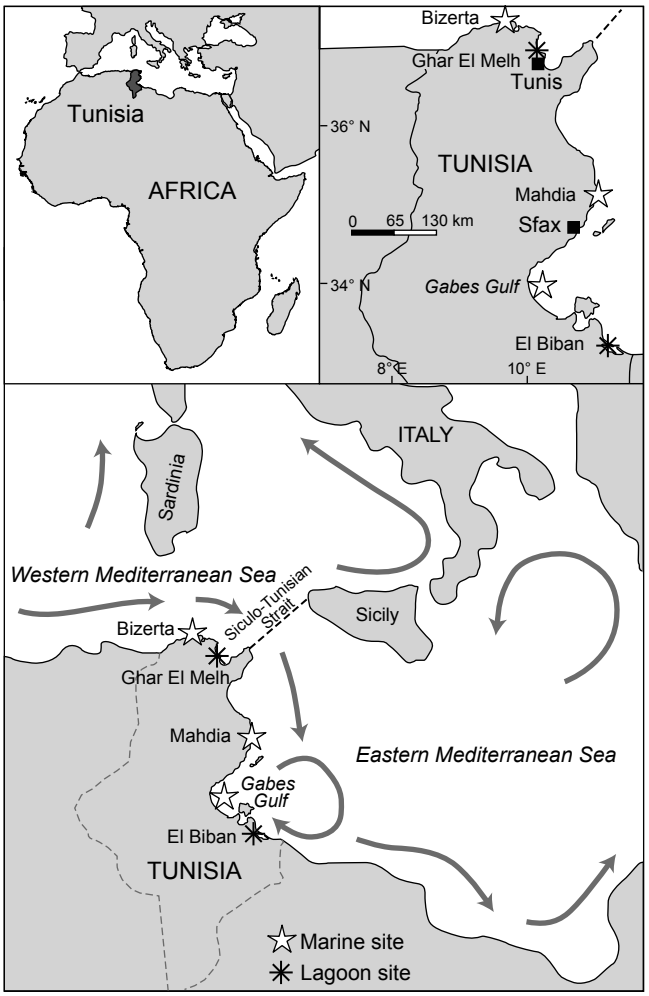

Figure 1: Map showing the location of the sampling sites along the Tunisian coast: Bizerta, Ghar El Melh Lagoon, Mahdia, Gabes Gulf and El Biban Lagoon. The Siculo-Tunisian strait lies between Cap Bon in Tunisia and Marsala in Sicily, Italy. The arrows indicate the current circulation

Polymerase chain reaction (PCR) cycles were conducted under the following conditions: 3 min at 94 °C, followed by 40 cycles of denaturing at 94 °C for 30 s, annealing at 56 °C for 30 s and extension at 72 °C for 2 min; finishing with an extension step at 72 °C for 5 min.

PCR products were electrophoresed and purified using ExoSAP-IT kit (USBEurope GmbH). After mixing the PCR products with ExoSap-IT and incubating at 37 °C, the ExoSAP-IT was inactivated by heating to 80 °C. Purified DNA was sequenced with an ABI sequencing kit (Big Dye Terminator Cycle Sequencing v. 2.0 ABI PRISM, Applied Biosystems) and then analysed with an ABI 3700 automated sequencer.

Data analysis

Allozyme data analysis

Genetic variability was estimated using observed (H_o) and unbiased expected (H_e) heterozygosities (Nei and Chesser 1983), mean number of alleles per locus (A_m) and number of exclusive alleles (A_e). The estimator *f* (Weir and Cockerham 1984) of Wright's fixation index (F_{IS}) (Wright 1969) was calculated in order to test departure from Hardy-Weinberg equilibrium (HWE). One thousand allelic permutations for each sample and each locus were performed. Loci contributing to departure from panmixia were analysed using jackknifing (Weir 1990). The estimator θ of F_{ST} according to Weir and Cockerham (1984) was calculated between populations to detect genetic differentiation. Significant deviations from the null hypothesis of genetic homogeneity were assessed by 1 000 permutations procedure of multilocus genotypes. These analyses were carried out using Genetix v.4.05 software (Belkhir et al. 2004).

To detect loci potentially under selection, we used the LOSITAN v.1.44 software (Antao et al. 2008), which evaluates the relationship between F_{ST} and expected heterozygosity (H_e), describing the expected distribution of F_{ST} vs H_e under an island model of migration with neutral markers (Beaumont and Nichols 1996). The distribution established after coalescent simulations was used to identify outlier loci that have excessively high or low F_{ST} values compared to neutral expectations. Such outlier loci are candidates for being subject to selection. We ran 50 000 simulations, starting with a neutral mean F_{ST} and using the entire dataset.

Co-ancestry genetic distances (*D*) (Reynolds et al. 1983) between pairwise samples were calculated and the corresponding matrix was used to perform a rooted tree using UPGMA (unweighted pair group method with arithmetic mean) clustering method (Sneath and Sokal 1973). The UPGMA tree is rooted using midpoint rooting. Node robustness was obtained from 1 000 bootstrap replicates. These analyses were performed using the phylogenetic package Phylip v.3.57 (Felsentein 1995).

Mitochondrial data analysis

DNA sequences were manually corrected with BioEdit v.5.0.6 software (Hall 1999) and aligned using Clustal X v.1.8 software (Thompson et al. 1994). Haplotype sequences were submitted to GenBank under the following accession numbers: GQ915000–GQ915015.

To evaluate the genetic diversity of populations, haplotype (H_d) and nucleotide (π) diversities were calculated using ARLEQUIN v.2.000 software (Schneider et al. 2000). The same program was used to calculate the pairwise F_{ST} values. Significance was performed using 10 000 permutations. The estimation of the Jukes-Cantor distance (Jukes and Cantor 1969) showed low values of *d*, ranging from 0.001 to 0.002. Moreover, the Kimura 2-parameter distances (Kimura 1980) assume that the four nucleotide frequencies are the same and that rates of substitution do not vary among sites. Those conditions allowed us to use the Kimura-2 parameter distances (Kimura 1980) which were computed in Mega v.4 (Tamura et al. 2007).

Intraspecific relationships were estimated using TCS v.1.13 software (Templeton et al. 1992, Clement et al. 2000). This method uses coalescence theory (Hudson 1991) to determine the limits of parsimony to define a set of plausible connections among haplotypes that have accumulative probability of >95% of being true (Templeton et al. 1992). This method is considered more appropriate than traditional

phylogenetic approaches for closely related sequences. It also provides a way to visualise alternative connections (i.e. 'loops') that are otherwise collapsed into unresolved polytomies (Posada and Crandall 2001).

Mismatch distribution for all samples (frequency of pairwise differences between haplotypes) was also carried out to explore the demographic history of Tunisian populations (Rogers and Harpending 1992). Expansion parameters (θ_0, θ_1 and τ) were calculated with the generalised non-linear least-square method (Schneider and Excoffier 1999). The time of possible population expansions (t) was calculated using the relationship $\tau = 2ut$ (Rogers and Harpending 1992), where τ is the mode of the mismatch distribution, u is the mutation rate of the sequence considering that $u = \mu k$ (μ is the mutation rate per nucleotide and k is the number of nucleotides), and t is the time of expansion. A conventional mutation rate for cytochrome *b* used in our analyses is of 0.94% per nucleotide per million years (Van Houdt et al. 2003). Mismatch distribution can show if a population has undergone a fast population expansion or has remained stable over time. Samples with a demographic equilibrium have usually a multimodal distribution; however, there will be an unimodal distribution if the population has a recent demographic expansion (Slatkin and Hudson 1991, Rogers and Harpending 1992, Excoffier 2004). Mismatch distribution significance was tested with the sum of square deviations (SSD) between observed and expected mismatch distributions. The *p*-value represents the probability of obtaining simulated SSD equal or larger than the observed one (Schneider and Excoffier 1999). ARLEQUIN v.2.000 software (Schneider et al. 2000) was used to test departures from mutation-drift equilibrium with Tajima's *D*-test (Tajima 1989). We also assessed the history of effective population size by means of other statistics such as Fu's *F* (Fu 1997), using DNA_{SP} v. 4.10.9 software (Rozas et al. 2003).

Results

Allozymes

In all, 13 polymorphic loci allowed the identification of 33 alleles (Table 1), and four unique alleles were detected

Table 1: Allele frequencies at each locus in the five Tunisian samples of *D. sargus*: sample size (*n*), number of alleles (*A*), observed heterozygosity (H_o), unbiased expected heterozygosity (H_e), Weir and Cockerham's (1984) fixation index (*f*), measuring departure from theoretical Hardy-Weinberg expectations for each locus and each sample are shown

Locus	Bizerta (*n* = 49)	Ghar Melh Lagoon (*n* = 25)	Mahdia (*n* = 35)	Gabes Gulf (*n* = 33)	El Biban Lagoon (*n* = 22)	Global/locus
*AAT-1** (*A* = 3)						
85	0.020	0.020	0.000	0.015	0.045	
100	0.970	0.920	0.956	0.985	0.955	
115	0.010	0.060	0.043	0.000	0.000	
H_e	0.059	0.149	0.082	0.029	0.086	
H_o	0.061	0.160	0.085	0.030	0.000	
f	−0.014^{ns}	−0.049^{ns}	−0.030^{ns}	−0.000^{ns}	1.000***	0.130^{ns}
*AAT-2** (*A* = 2)						
100	1.000	1.000	1.000	0.967	1.000	
110	0.000	0.000	0.000	0.030	0.000	
H_e	0.000	0.000	0.000	0.058	0.000	
H_o	0.000	0.000	0.000	0.000	0.000	
f	–	–	–	1.000**	–	1.000**
*EST-1** (*A* = 4)						
90	0.000	0.060	0.071	0.000	0.000	
100	0.745	0.880	0.800	0.743	0.932	
110	0.204	0.060	0.129	0.242	0.023	
120	0.051	0.000	0.000	0.015	0.045	
H_e	0.400	0.218	0.338	0.389	0.129	
H_o	0.122	0.160	0.285	0.212	0.136	
f	0.610***	–	–	0.468***	−0.024^{ns}	0.442***
*EST-2** (*A* = 3)						
90	0.000	0.040	0.000	0.182	0.160	
100	0.990	0.920	0.914	0.803	0.840	
110	0.010	0.040	0.086	0.015	0.000	
H_e	0.020	0.150	0.156	0.321	0.267	
H_o	0.020	0.080	0.000	0.030	0.045	
f	−0.000^{ns}	0.484***	1.000***	0.909***	0.837**	0.818***
*EST-3** (*A* = 2)						
100	1.000	1.000	1.000	0.940	1.000	
115	0.000	0.000	0.000	0.060	0.000	
H_e	0.000	0.000	0.000	0.114	0.000	
H_o	0.000	0.000	0.000	0.000	0.000	
f	–	–	–	1.000**	–	1.000***

(Table 2). Three of them were found in the sample of Gabes Gulf (*SOD*160*; *AAT-2*110* and *EST-3*115*) and one in El Biban Lagoon (*ICD-1l*80*). Diversity parameters showed that the highest values of average number of alleles (A_m = 1.812) and average expected heterozygosity (H_e = 0.103 ± 0.045) were observed in the sample of Gabes Gulf (Table 2). Global F_{IS} value, used for testing Hardy-Weinberg equilibrium, was high and significant (global f = 0.506; $p < 0.001$), indicating that populations are not in equilibrium. The jackknife resampling procedure allowed

Table 1 (cont.)

Locus	Bizerta (n = 49)	Ghar Melh Lagoon (n = 25)	Mahdia (n = 35)	Gabes Gulf (n = 33)	El Biban Lagoon (n = 22)	Global/locus
*ICD-1m** (A = 2)						
80	0.000	0.000	0.000	0.046	0.068	
100	1.000	1.000	1.000	0.954	0.932	
H_e	0.000	0.000	0.000	0.087	0.127	
H_o	0.000	0.000	0.000	0.091	0.045	
f	–	–	–	–	−0.000^{ns}	0.007^{ns}
*ICD-1l** (A = 2)						
80	0.000	0.000	0.000	0.000	0.023	
100	1.000	1.000	1.000	1.000	0.977	
H_e	0.000	0.000	0.000	0.000	0.044	
H_o	0.000	0.000	0.000	0.000	0.045	
f	–	–	–	−0.032^{ns}	0.656^{ns}	0.307^{ns}
*ICD-2** (A = 2)						
80	0.000	0.040	0.029	0.000	0.068	
100	1.000	0.960	0.971	1.000	0.932	
H_e	0.000	0.077	0.055	0.000	0.127	
H_o	0.000	0.000	0.000	0.000	0.045	
f	–	1.000*	1.000*	–	0.656^{ns}	0.854***
*MDH-1** (A = 2)						
80	0.153	0.180	0.157	0.121	0.227	
100	0.847	0.820	0.843	0.879	0.773	
H_e	0.259	0.295	0.264	0.213	0.351	
H_o	0.102	0.200	0.142	0.121	0.181	
f	0.613***	0.341^{ns}	0.472*	0.443^{ns}	0.500*	0.490***
*PGI-1** (A = 3)						
80	0.010	0.080	0.000	0.000	0.000	
100	0.990	0.900	0.986	1.000	0.977	
120	0.000	0.020	0.014	0.000	0.023	
H_e	0.020	0.183	0.028	0.000	0.044	
H_o	0.020	0.200	0.028	0.000	0.045	
f	−0.000^{ns}	−0.071^{ns}	0.000^{ns}	–	−0.000^{ns}	−0.046^{ns}
*PGI-2** (A = 3)						
80	0.000	0.020	0.000	0.015	0.000	
100	0.980	0.980	0.986	0.955	0.977	
120	0.020	0.000	0.014	0.030	0.023	
H_e	0.040	0.039	0.028	0.087	0.044	
H_o	0.041	0.040	0.028	0.090	0.045	
f	−0.011^{ns}	−0.000^{ns}	0.000^{ns}	−0.021^{ns}	−0.000^{ns}	−0.010^{ns}
*PGM** (A = 3)						
90	0.000	0.020	0.014	0.000	0.205	
100	0.939	0.980	0.986	0.820	0.795	
110	0.061	0.000	0.000	0.180	0.000	
H_e	0.115	0.039	0.028	0.297	0.325	
H_o	0.000	0.040	0.028	0.060	0.136	
f	1.000***	−0.000^{ns}	0.000^{ns}	0.802**	0.596*	0.722***
*SOD** (A = 2)						
100	1.000	1.000	1.000	0.985	1.000	
160	0.000	0.000	0.000	0.015	0.000	
H_e	0.000	0.000	0.000	0.000	0.000	
H_o	0.000	0.000	0.000	0.000	0.000	
f	–	–	–	−0.000^{ns}	–	0.0001^{ns}

ns = not significant
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
m = muscle; l = liver

Table 2: Genetic diversity parameters in *D. sargus* samples from the Tunisian coasts: sample size (n), average observed heterozygosity (H_o), average unbiased expected heterozygosity (H_e), mean number of alleles per locus and per population (A_m), number of exclusive alleles (A_e), Weir and Cockerham's (1984) multilocus fixation index (multilocus f) measuring departure from theoretical Hardy-Weinberg expectations, number of haplotypes (h), segregating sites (S), haplotype diversity (H_d), nucleotide diversity (π)

Sampling site	Allozymes						mtDNA				
	n	H_o	H_e	A_m	A_e	Multilocus f	n	h	S	H_d	π
Bizerta	49	0.023 ± 0.011	0.057 ± 0.032	1.562	–	0.605***	19	5	4	0.578	0.0010
Ghar El Melh Lagoon	25	0.055 ± 0.03	0.073 ± 0.038	1.750	–	0.255***	8	4	3	0.785	0.0018
Mahdia	35	0.037 ± 0.025	0.062 ± 0.034	1.562	–	0.401***	25	8	8	0.77	0.0017
Gabes Gulf	33	0.041 ± 0.021	0.103 ± 0.045	1.812	3	0.600***	7	5	4	0.857	0.0017
El Biban Lagoon	22	0.045 ± 0.025	0.099 ± 0.051	1.687	1	0.546***	7	6	5	0.952	0.0024
Total	164	0.044 ± 0.007	0.081 ± 0.015	1.687	4	0.506***	66	16	16	0.735	0.0016

*** $p < 0.001$

us to calculate a standard deviation of global f-values over loci (global F_{IS} = 0.506 ± 0.073). Single locus f-values for all samples (Table 1) showed departure from HWE at seven loci out of 13 (*AAT-2**, *EST-1**, *EST-2**, *EST-3**, *ICD-1m**, *MDH-1** and *PGM**). Heterozygosity deficit was also observed in each sample (Table 2).

Global F_{ST} value was significant (F_{ST} = 0.027; $p < 0.001$), showing the existence of differentiation among localities. Pairwise comparisons showed significant values at 0.05 and 0.01 levels (Table 3) between some north-eastern samples and southern ones. However, only the F_{ST} value between the Bizerta marine sample (north-eastern coast) and El Biban Lagoon (southern coast) remained significant after the sequential Bonferroni correction (Table 3). Global F_{ST} values estimated for each locus ranged between 0 and 0.089, which allowed us to distinguish two groups of loci (Figure 2) that have not the same contribution to observed genetic differentiation. The first group was composed of seven loci (*MDH-1**, *PGI-2**, *AAT-1**, *AAT-2**, *SOD**, *ICD-2** and *ICD-1l**), which showed low and non-significant F_{ST} values. The second grouped the six other loci, which exhibited significant F_{ST} values and moderate (*EST-1**, *EST-3**, *ICD-1m**) to high differentiation (*EST-2**, *PGI-1**, *PGM**).

Coalescent simulations (performed by LOSITAN software), using overall F_{ST} (F_{ST} = 0.028), as an expected value for neutral markers and infinite allele model, found the upper and lower F_{ST} limits at the 0.95 probability level (Figure 3). Among the 13 polymorphic loci, *PGM** and *PGI-1** appeared to be candidates for selection (Figure 3). *PGM** locus was a candidate to positive directional selection (H_e = 0.183, F_{ST} = 0.105, p = 0.987), whereas *PGI-1** (H_e = 0.048, F_{ST} = −0.008, p = 0.038) was considered as a balancing selection candidate.

Phylogenetic trees based on co-ancestry genetic distances drawn with all loci (Table 3 and Figure 4) or without those potentially under selection (tree not shown) showed the same topology with two clusters. The first group was made up of Bizerta marine, Ghar El Melh Lagoon and Mahdia samples. The second group included the two southern samples: Gabes Gulf and El Biban Lagoon. The bootstrap values were moderate in both cases (73.5% with all loci; 57.8% without loci potentially under selection).

Table 3: Co-ancestry genetic distance values (Reynolds et al. 1983) (above diagonal) and θ values (below diagonal), estimator of F_{ST} according to Weir and Cockerham (1984) among *D. sargus* samples for allozyme data. Underlining denotes significant values after sequential Bonferroni correction (α' = 0.005)

θ \ D	Bizerta	Ghar El Melh Lagoon	Mahdia	Gabes Gulf	El Biban Lagoon
Bizerta		0.018	0.001	0.027	0.065
Ghar El Melh Lagoon	0.018^{ns}		−0.007	0.041	0.018
Mahdia	0.001^{ns}	0.000^{ns}		0.035	0.042
Gabes Gulf	0.026*	0.041*	0.035*		0.033
El Biban Lagoon	<u>0.063</u>**	0.018^{ns}	0.041*	0.032*	

* $p < 0.05$, ** $p < 0.01$
ns: not significant

mtDNA

A total of 671 bp was generated for the cytochrome *b* region for 66 individuals. In all, 16 nucleotide sites were variable, five nucleotide sites were parsimony informative and 11 were autapomorphic. The transition/transversion ratio was high (R = 17.255). Among these sequences, 16 haplotypes were identified. The highest number of haplotypes (eight) was found in Mahdia and the lowest (four) was detected in Ghar El Melh Lagoon.

The Bizerta sample showed the lowest haplotype and nucleotide diversities (H_d = 0.578; π = 0.001). However, the southern lagoon sample (El Biban) exhibited the highest haplotype and nucleotide diversities (H_d = 0.952; π = 0.002) (Table 2).

The Kimura 2-parameter distances were low and ranged from 0.002 (Bizerta and Gabes Gulf) to 0.003 (Mahdia and El Biban Lagoon). F_{ST} values ranged from 0 (Mahdia and Gabes Gulf) to 0.029 (Ghar El Melh Lagoon and Gabes Gulf). These F_{ST} values were low and non-significant.

The statistical parsimony procedure yielded one network with several connections (Figure 5). The network had a star-like pattern in which the most common and ancestral haplotype (Cytb-1) presented a central position. Five

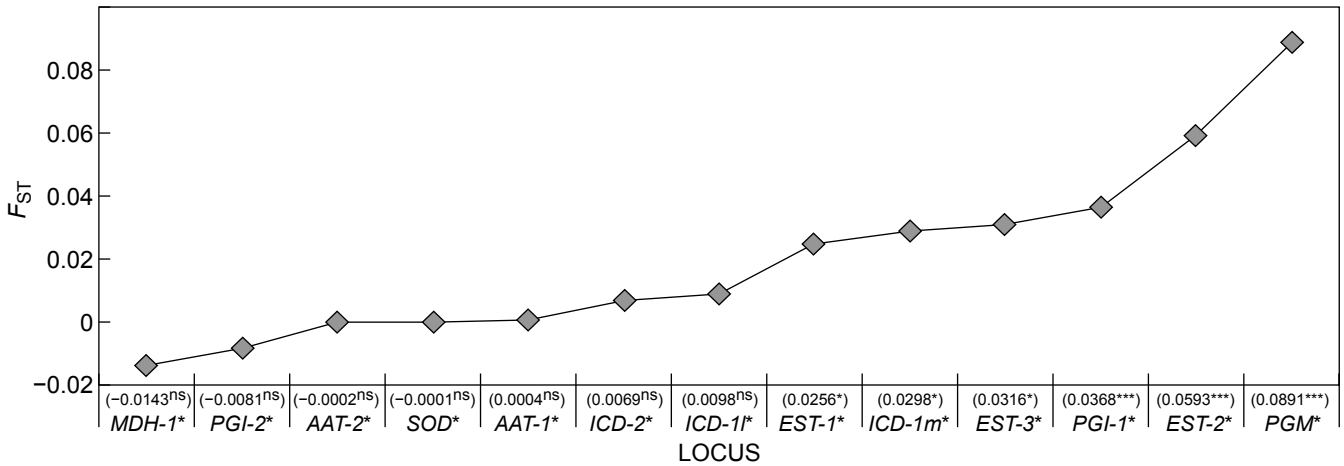

Figure 2: Global F_{ST} values among all samples and for each locus; θ-values in parenthesis above the loci; ns denotes not significant, * $p < 0.05$, *** $p < 0.001$

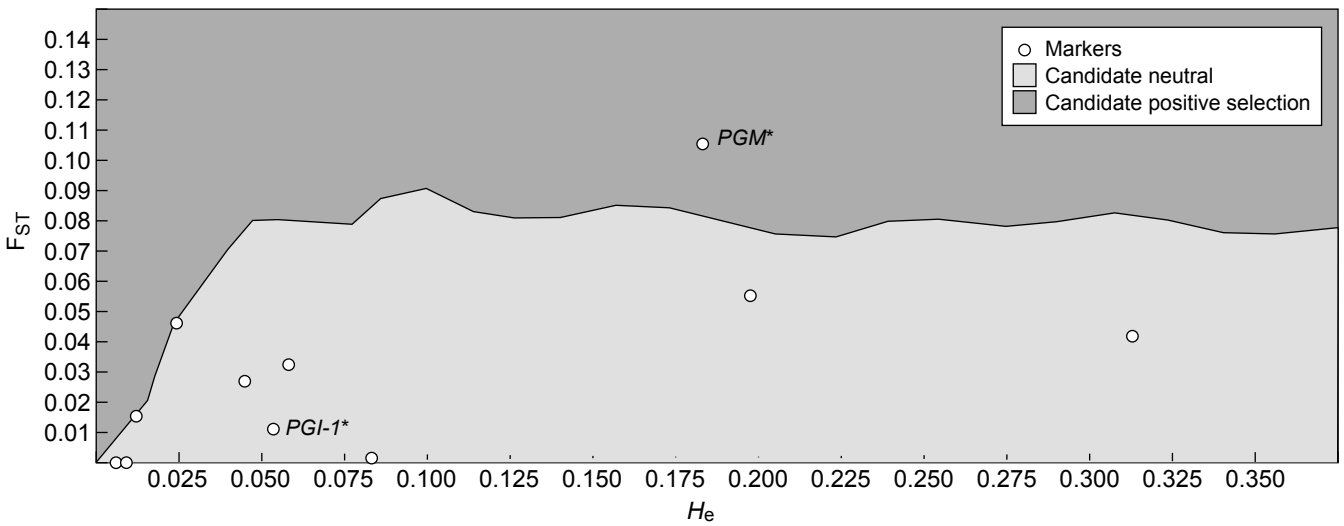

Figure 3: Comparison of F_{ST} and H_e in polymorphic loci to identify outliers and potential candidates for selection using LOSITAN software (Antao et al. 2008). Graphical output shows the simulated confidence area for neutral loci (pale grey shading). Loci outliers are tagged with labels. Locus *PGM** is a candidate for positive selection, whereas *PGI-1** locus is a candidate for balancing selection (refer to the text)

haplotypes were separated by two mutational steps from the common haplotype (Cytb-9, Cytb-10, Cytb-11, Cytb-13 and Cytb-15) and the rest showed only one mutational step.

Because F_{ST} values among sampled populations were not significant, they were grouped to the mismatch distribution analysis. The unimodal mismatch distribution obtained (Figure 6) and the star-like phylogeny of haplotypes (Figure 5) suggested a population expansion. Moreover, the SSD based on 1 000 replicates for simulated datasets, was larger than the SSD for observed data (Figure 6), the negative and significant D (−2.009; $p = 0.010$) and F_S (−13.3; $p = 0.000$) values are consistent with a scenario of a sudden demographic expansion of the *D. sargus* population. This expansion event was estimated to take place 85.86 generations ago. Given that *D. sargus* reaches full sexual maturity at 2–3 years (Lenfant 1998, Lloret and Planes 2003), the expansion time of the Tunisian *D. sargus* population would thus have occurred approximately 172 thousand years ago.

Discussion

Genetic diversity

Allozyme data from Tunisian samples showed a low level of genetic diversity (A_m ranged from 1.562 to 1.812 and H_e ranged from 0.057 to 0.103). These values are similarly reflected in genetic diversity parameters observed in populations from the Ligurian Sea and the Gulf of Lion ($A_m = 1.64$ and $H_e = 0.144$; Lenfant and Planes 1996) and from several Atlanto–Mediterranean populations (A_m ranging from 1.368 to 1.473 and H_e ranging from 0.077 to 0.088; Bargelloni et al. 2005). Similar low levels of genetic diversities

were reported for other sparid species such as *Diplodus puntazzo* (H_e ranging from 0.028 to 0.050; Bargelloni et al. 2005), *Diplodus vulgaris* (H_e = 0.093; Arculeo et al. 2003), *Lithognathus mormyrus* (H_e = 0.072; Arculeo et al. 2003, H_e ranging from 0.006 to 0.017; Bargelloni et al. 2003, H_e = 0.087; Hammami et al. 2007), *Pagrus pagrus* (H_e ranging from 0.053 to 0.107; Bargelloni et al. 2003), and *Pagellus erythrinus* (H_e ranging from 0.023 to 0.1; Fassatoui et al. 2009).

The levels of genetic diversity described for south-western Spanish Mediterranean samples (A_m ranging from 5 to 5.57 and H_e from 0.549 to 0.577; González-Wangüemert et al. 2004, 2006) were higher than those obtained in the present study. These large differences could be associated with the inclusion of marine protected areas in the abovementioned studies, in which the genetic diversity is unusually high because of the 'protection effect' (Pérez-Ruzafa et al. 2006).

Mitochondrial analysis showed high haplotype diversity associated with low nucleotide diversity. Considering the Atlantic and western Mediterranean samples analysed by González-Wangüemert et al. (2010), Tunisian samples share the same range of haplotype (0.551–0.889) and nucleotide (0.001–0.003) diversities, except for those from El Biban Lagoon sample which showed a very high haplotype diversity (H_d = 0.95). However, it is important to note the high number of haplotypes detected in the Tunisian samples (16) with respect to previous data, for which 23 haplotypes were only

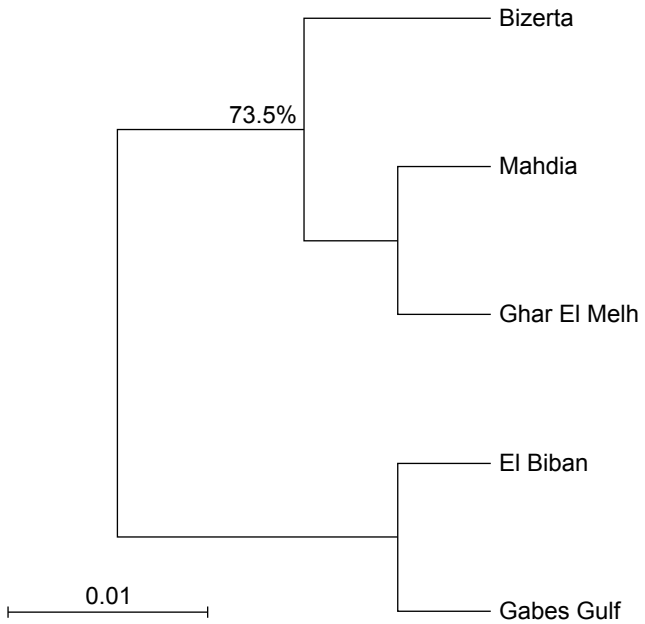

Figure 4: Cluster analyses by UPGMA of six samples of *Diplodus sargus* using the co-ancestry genetic distance (Reynolds et al. 1983) calculated from allozyme data. The bootstrap value is calculated with all loci

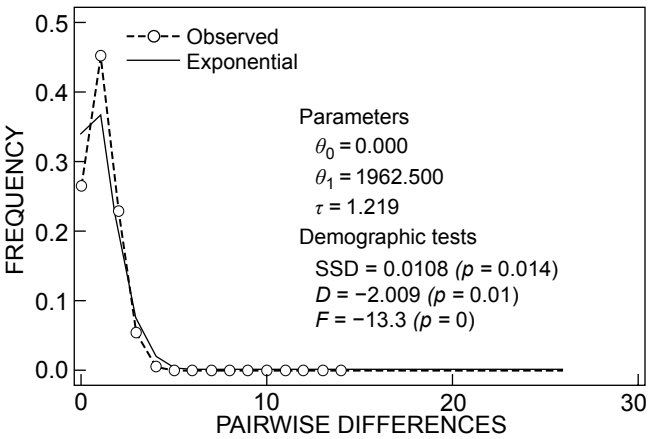

Figure 6: Pairwise mismatch distribution of haplotypes of *D. sargus* for mtDNA cytochrome *b*. SSD = sum of square deviations between observed and expected mismatch distributions; *p* = probability of obtaining simulated SSD equal or larger than the observed one; *D* = Tajima's *D*-test, *F* = Fu's test. Expansion parameters: initial theta (θ_0), final theta (θ_1) and generation time (τ)

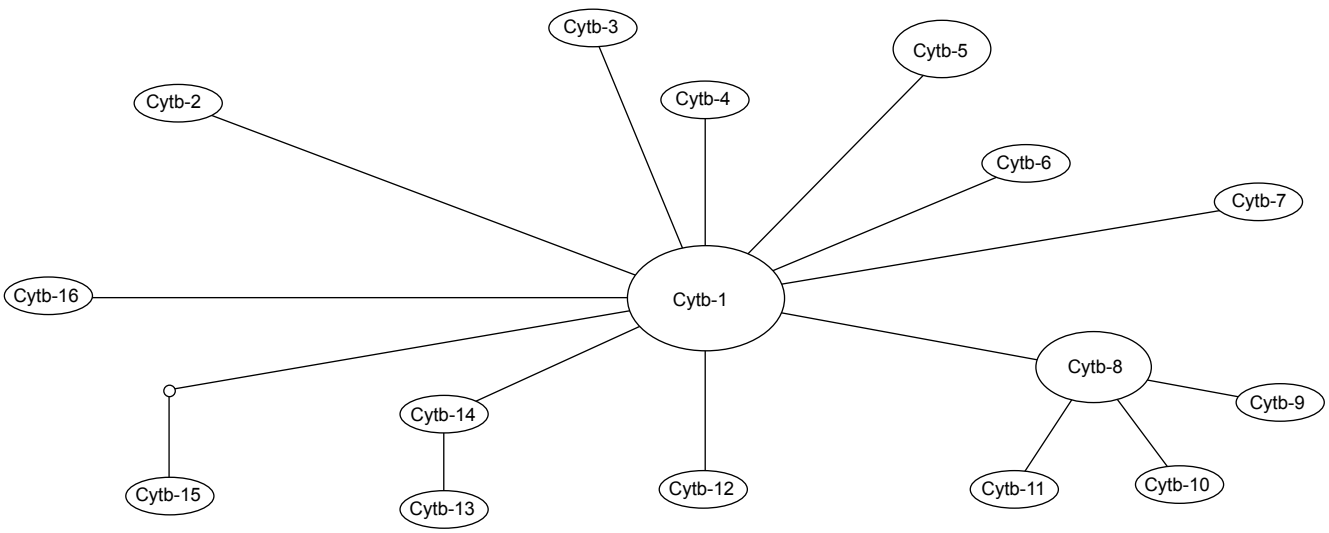

Figure 5: Statistical parsimony network based on the cytochrome *b* sequences of Tunisian *D. sargus*

described, but covering a large geographic area (González-Wangüemert et al. 2010). This feature of Tunisian samples can be explained by the number of exclusive haplotypes found in Mahdia and El Biban Lagoon.

All Tunisian samples showed high and significant F_{IS} values (F_{IS} ranging from 0.24 to 0.446), reflecting an important departure from Hardy-Weinberg equilibrium. Similar departure was observed by Lenfant and Planes (1996) in the Gulf of Lion and Ligurian Sea (F_{IS} ranging from 0.014 to 0.126). Similarly, high F_{IS} values ranging between 0.329 and 0.446 were reported for the *D. sargus* populations from the south-east of Spain (González-Wangüemert et al. 2004), which were mainly explained by the Whalund effect. However, the study of Bargelloni et al. (2005) in the Atlanto–Mediterranean region using the same markers did not show the same departure from Hardy-Weinberg equilibrium. Deficit of heterozygotes is common in marine fish (Garcia de Leon 1995, Allegrucci et al. 1997, Lundy et al. 1999) and can be explained, in the case of Tunisian *D. sargus* populations, by several hypotheses such as null alleles, artefactual factors related to staining, the Wahlund effect, selection against heterozygotes, or a combination of several factors. Among these hypotheses, the Wahlund effect seems to be the most reasonable as a consequence of the planktonic mixing of larvae, as demonstrated by Lenfant and Planes (2002) and Planes and Lenfant (2002). These authors considered that individuals in a cohort are the result of mixing the output of several families, among which — especially reduced ones — genetic drift has occurred. This hypothesis was advanced to explain heterozygote deficit in some Mediterranean *D. sargus* populations (Lenfant and Planes 1996, González-Wangüemert et al. 2004).

Another hypothesis that could explain the deficit of heterozygotes is the selection against heterozygotes. Such selective processes that, described in *D. sargus* species, can limit the survival rate and affect the allelic frequencies of different cohorts (Planes and Romans 2004). This hypothesis has been advanced for many marine fish species such as Dover sole *Solea vulgaris* (Kotoulas et al. 1995) and sea bass *Dicentrarchus labrax* and *D. punctatus* (Allegrucci et al. 1997).

Discrepancy in mtDNA and allozyme differentiation

In our study, nuclear and mitochondrial markers gave conflicting results. Moderate heterogeneity was found between samples from allozyme data, which did not seem to be associated to any genetic transition through the Siculo-Tunisian Strait. However, mitochondrial DNA data demonstrated the lack of genetic structure in *D. sargus* samples along the Tunisian coasts. The most likely hypothesis to explain microgeographical genetic heterogeneities using allozyme markers is the presence of chaotic genetic patchiness in large panmictic populations (Johnson and Black 1982). Planktonic dispersal, causing generally uniformity on a large scale, can sometimes give rise to fine-scale genetic patchiness. Thus, mixing differentiated larval pools can be the cause of temporal variations in the genetic composition of recruits, resulting in chaotic genetic patchiness (Planes and Lenfant 2002). In some cases, populations separated by <10–100 km can be genetically different from populations separated by 100–1 000 km (Johnson and Black 1982, Larson and Julia 1999), as has been reported for *D. sargus* populations (Lenfant and Planes 2002, Planes and Lenfant 2002, Pérez-Ruzafa et al. 2006, González-Wangüemert et al. 2007). Such unpatterned genetic heterogeneity among local populations has been also found in some marine invertebrates (Johnson and Black 1982, 1984, Watts et al. 1990, Johnson et al. 1993) and other fish (Fauvelot and Planes 2002). Theoretically, the planktonic mixing of larvae could favour the Wahlund effect, whereas the gene flow caused by planktonic dispersal should counter that effect by decreasing genetic differentiation among adult populations (Lenfant and Planes 2002).

Our results show that *PGI-1** and *PGM** loci can be considered candidates of natural selection or closely linked to selected gene. In fact, enzyme loci can provide high differentiation in relation to ecological or biological features such as average size, growth rates, sexual maturity, and behaviour (Wilson and Clarke 1996, Allegrucci et al. 1997, Lemaire et al. 2000). Planes and Romans (2004) demonstrated relationships between growth selection pattern in *D. sargus* and *PGM* variability. Selection factors were also hypothesised to explain variations of *PGI* loci in some marine fish such as Atlantic cod *Gadus rnorhua* (Mork and Sundnes 1985), the European hake *Merluccius merluccius* (Lo Brutto et al. 1998, 2004) and the marine goby *Pomatoschistus lozanoi* (Gysels et al. 2004). González-Wangüemert et al. (2009) showed that environmental conditions (salinity, sediment type and local current patterns) of the Mar Menor coastal lagoon in Spain can have an influence on the *PGI* genetic variability of the bivalve *Cerastoderma glaucum*. Nevertheless, in terms of gene flow, the selection hypothesis cannot explain heterogeneity observed with allozyme data. This is justified by the tree topology which remains with the same shape/distribution. Such finding supports the chaotic genetic patchiness hypothesis.

On the other hand, mitochondrial data showed a high gene flow between localities. Coastal habitat continuity between sites and larvae dispersal duration (3–4 weeks) favour a high genetic connectivity, which can explain the detected homogeneity. Juvenile and adult phases of white seabream are active swimmers and this feature could also allow the rapid mixing between samples from both basins. According to these results, the Siculo-Tunisian Strait does not limit the gene flow between northern and southern *D. sargus* populations and therefore it does not act as a real biogeographical barrier.

These contrasting signals complicate the reconstruction of connectivity of studied populations. Such situations are not unusual as several studies have shown dissimilar patterns (Lemaire et al. 2005, Gonzalez and Zardoya 2007, Sala-Bozano et al. 2009). Based on the present data, it is unclear as to which structure would be appropriate. Homogeneity reported with mtDNA seems to be the correct structure of populations in this transitional area, whereas allozyme data may be affected mainly by stochastic, cohort-based events typical from a chaotic genetic patchiness. Such Wahlund effect has been extensively reported for *D. sargus* (Planes and Lenfant 2002, González-Wangüemert et al. 2007). Nevertheless, it would be interesting to use other markers to provide a comprehensive picture of geographic population structure of *D. sargus* populations in this transitional area.

Historical demography

Historical demography of Tunisian *D. sargus* populations based on the mitochondrial cytochrome *b*, according to the mismatch distribution, showed a sudden expansion of their population size. The estimated expansion time of around 172 thousand years ago, associated with results of mismatch distribution, suggests that this species underwent a recent expansion as a consequence of a bottleneck event during the Pleistocene glaciations (Lambek et al. 2002). This bottleneck probably created a strong reduction in the size of the population, which could explain the lack of genetic differentiation in this species (Bargelloni et al. 2005). This recent population expansion in the western and central Mediterranean Sea was shown by Bargelloni et al. (2005) using control region marker, and later confirmed by González-Wangüemert et al. (2010, 2011) using cytochrome *b* and control region markers. Domingues et al. (2007) used control region markers to show a clear population expansion only in the Eastern Mediterranean basin only (Greek islands), but not for the Western basin (Barcelona).

Acknowledgements — Financial support was provided by the international programme of scientific and investigation of Spanish–Tunisian cooperation, and from the University of Science of Tunis. Partial financial support was received from the SENECA Program (03000/PI/05) and the AECI Program (Agencia Española de Cooperación Internacional; Ministerio de Asuntos Exteriores, A/4396/05-A/6704/06). MGW was supported by a FCT (Fundação para a Ciência e a Tecnologia) postdoctoral grant (SFRH/BPD/70689/2010). Thanks are due to Samia Kort for linguistic assistance. We are also grateful to two anonymous reviewers who helped to improve the quality of the manuscript, through their suggestions and constructive comments.

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