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Genetic differentiation of *Diplodus sargus* (Pisces: Sparidae) populations in the south-west Mediterranean

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Allozyme analysis of tissue samples of 1249 white sea bream *Diplodus sargus* from five localities of the south-west Mediterranean revealed a high degree of genetic polymorphism. The observed heterozygosity ranged from 0.4182 (Cape of Palos) to 0.3138 (Tabarca). Several populations were characterized by unique alleles. Examination of the spatial structure was performed using Nei's distances and *F*-statistics, and indicated genetic differences between groups. One group, which clustered Tabarca and Guardamar, could be explained by the small geographical distance between them. Mazarrón and Cape of Palos samples showed genetic divergence from other samples (Guardamar, Tabarca and Águilas) and this difference may be as a result of local current systems and larval dispersal. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, **82**, 249–261.

ADDITIONAL KEYWORDS: allozyme – gene pool – genetic structure – Mediterranean Sea.

INTRODUCTION

The white sea bream *Diplodus sargus* L. is a commercial species found throughout the eastern temperate Atlantic and Mediterranean Seas (Fisher, Schneider & Bauchot, 1987; Lenfant & Planes, 1996a), where it occurs in coastal rocky reef areas and *Posidonia* beds. Two subspecies have been reported (Bauchot & Hureau, 1986), *D. s. cadenati* in the Atlantic coasts, from Bay of Biscay to Cape Verde, and *D. s. sargus* from the Mediterranean Sea to the Black Sea. Adults spawn in the open sea from March to June (Leboulleux, 1992) and eggs are reported to occur in the surface waters to a depth of 5 m (Marinaro, 1971), the larvae spending 3–4 weeks in the open sea before reaching favourable sites for recruitment (Vigliola, 1998). Settlement of *D. sargus* takes place at very shallow depths (0–2 m) on sandy–rocky bottoms in May–June (Garcia-Rubies & Macpherson, 1995; Harmelin-Vivien, Harmelin & Leboulleux, 1995). The juveniles remain in the vicinity of the settlement sites for 2 or 3 months until they reach 4.5–5.5 cm in length (Macpherson, 1998).

Due to their importance, considerable research has been devoted to the study of their biology, ethology and growth in Mediterranean waters (Wassef, 1985; Abou-Seedo, Wright & Clayton, 1990; Micale & Perdichizzi, 1994; Macpherson *et al*., 1997). However, knowledge of their population structure and dynamics and connectivity between populations in the context of metapopulations (Hauski, 1999) is still limited.

The most effective means of elucidating population structure is by genetically comparing fish from a number of locations. In this regard the technique of allozyme electrophoresis has been used extensively as a source of genetic markers for investigations of stock structure characteristics of important commercial and recreational fish species (Smith, Jamieson & Birley, 1990; Smith, 1990; Utter, 1991; Carvalho, 1993; Ward & Grewe, 1994).

These genetic markers, used to discriminate two or more populations, need to be sufficiently polymorphic to allow differences between the populations to have

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arisen; if the marker is monomorphic it will not be possible to elucidate population structure (Chikhi, Bonhomme & Agnese, 1998).

In fact, allozymes not only provide estimators of genetic differentiation and reproductive isolation, but also provide data on mating patterns in relation to the Hardy–Weinberg equilibrium. It is thereby possible to determine whether individuals in a sample are drawn from a large, randomly mating population with equilibrium genotype frequencies, or whether samples comprise an assemblage of genetically distinct units (Mamuris, Apostolidis & Trianta-Phyllidis, 1998), assuming the markers are selectively neutral. Such data are valuable for stock structure analysis and conservation of genetic resources, for which the extent of fisheries activities (harvesting, size-selective mortality) and the genetic component of population differentiation are important management considerations (Carvalho & Hauser, 1994).

The first allozyme studies on the genetic structure of *D. sargus* were based on few loci and individuals; little information concerning structure and genetic variability was available (Vazzana *et al*., 1990). Since then, several studies on enzymatic variability of *D. sargus* have been published (Basaglia & Marcheti, 1990; Santulli *et al*., 1991; Reina *et al*., 1994; Lenfant & Planes, 1996a, b; Cervelli, 1998, 1999). Some have shown significant differentiation between populations. Lenfant (1998) demonstrated that *D. sargus* samples from the Lion's Gulf and the Ligurian Sea are not genetically homogeneous. Also, significant changes in the genetic structure within and between year classes have been detected in *D. sargus* populations (Planes & Lenfant, 2002; Lenfant & Planes, 2002).

The Cape of Palos area studied in this work is considered a faunistic transitional zone between the Atlantic influence and the proper Mediterranean Sea. A number of Atlantic species occur in the area (Whitehead *et al*., 1986; J. A. García-Charton & Á. Pérez-Ruzafa, unpubl. data). We report here an estimate of the genetic intrapopulation variability and the degree of allozyme differentiation for samples of *D. sargus* taken from five localities of the south-western Mediterranean (Spain). We compare south-western data with the previous allozyme studies of *D. sargus* from the north-western Mediterranean (Lenfant, 1998).

MATERIAL AND METHODS

SAMPLING

A total of 1249 specimens of *D. sargus* (Linné, 1758) (Table 1), were captured between 1999 and 2000 from the eastern Spanish Mediterranean. Five localities were sampled: two marine reserves, Tabarca island (established in 1986) and Cape of Palos-Hormigas islands (established in 1995), along with three neighbouring non-protected areas (Águilas, Mazarrón and Guardamar) (Fig. 1). The number of sampled specimens at each locality ranged from 158 to 293 (Table 1).

Fish were frozen after capture, transported to the laboratory and kept at –40°C until dissection. Liver, eye and muscle were extracted from each specimen, and kept at –70°C until analysed.

ELECTROPHORESIS

Samples were homogenized in an equal volume of Tris-citrate buffer (pH 9). The homogenates were centrifuged at 7000 *g* for 12 min at 4 °C. The supernatant was stored at –80°C. Electrophoresis was performed on starch gel using six buffers (Tris-borate EDTA 8.6 (TBE); Tris-citrate 8.0 (TC); Tris-citrate-EDTA 7.4 (TCE); Tris-maleate 7.4 (TM); Tris-HCl 8.6; phosphate 7.0) according to Harris & Hopkinson (1976) and Aebersold *et al*. (1987). The following 14 loci were screened (nomenclature according to Shakle *et al*., 1990): glucose phosphate isomerase, EC 5.3.1.9 (*GPI-1** and *GPI-2**; TM 7.4 on muscle); phosphoglucomutase, EC 5.4.2.1 (*PGM**; TM 7.4 on muscle); phosphogluconate dehydrogenase, EC 1.1.1.44 (*PGDH**; TEB 8.6 on muscle); lactate dehydrogenase, EC 1.1.1.27 (*LDH-1** and *LDH-2**; phosphate 7.0 on muscle and liver, respectively); adenosine deaminase, EC 3.5.4.4 (*ADA**; TC 8.0 on liver); guanine deaminase, EC 3.5.4.3 (GDA*; Tris-HCl 8.6 on liver); isocitrate dehydrogenase EC 1.1.1.42 (*IDHP-1** and *IDHP-2**; TCE 7.0 on liver and muscle, respectively); aspartate amino transferase EC 2.6.1.1 (*AAT-1** and *AAT-2**; TBE 8.6 on muscle and liver, respectively); malate dehydrogenase EC 1.1.1.37 (*MDH-1** and *MDH-2**; TM 7.4 on muscle and liver, respectively).

The staining protocols followed Harris & Hopkinson (1976) and Aebersold *et al*. (1987).

Isozymes coded by separate loci were numbered in order of decreasing anodal mobility. The most common allele was given the value 100, and slower or faster bands, representing other alleles, were given lower or higher numbers, respectively (Mamuris *et al*., 1998).

POPULATION GENETIC ANALYSIS

Genetic variability was recorded as: observed (H_o) and expected (H_e) mean heterozygosity, proportion of polymorphic loci at the 5% and 1% criterion (P), and mean number of alleles per locus (A).

Genotypic frequencies were tested for conformity with Hardy–Weinberg expectations. Probabilities of random departure from Hardy–Weinberg equilibrium and significance of divergence among groups were calculated using the exact test method (Raymond & Rousset, 1995b).

Table 1. Allele frequencies for five populations of *Diplodus sargus*

Locus	Allele	Águilas (N = 262)	Cape of Palos (N = 269)	Guardamar (N = 158)	Mazarrón (N = 267)	Tabarca (N = 293)
*GPI-1**	40	0.28	0.27	0.21	0.24	0.28
	60	0.02	0.01	0.09	0.00	0.02
	80	0.02	0.00	0.04	0.00	0.02
	100	0.55	0.59	0.55	0.54	0.54
	120	0.01	0.00	0.01	0.00	0.00
	140	0.05	0.05	0.05	0.05	0.05
	160	0.07	0.09	0.04	0.15	0.07
	180	0.01	0.00	0.00	0.00	0.00
	200	0.00	0.00	0.00	0.01	0.02
*GPI-2**	40	0	0	0	0	0.04
	60	0.06	0	0.10	0.01	0.08
	80	0.19	0.20	0.08	0.20	0.16
	93	0.01	0	0.03	0.02	0.03
	100	0.58	0.60	0.59	0.62	0.57
	120	0.12	0.13	0.12	0.14	0.05
	140	0	0.01	0.05	0	0.08
	167	0	0.05	0.03	0.02	0.01
	180	0.03	0	0.01	0	0.01
*PGM**	40	0.16	0.15	0.19	0.21	0.19
	60	0.20	0.20	0.23	0.17	0.16
	100	0.44	0.44	0.41	0.47	0.49
	120	0.19	0.21	0.16	0.15	0.16
*PGDH**	40	0.02	0.04	0.08	0.01	0.04
	60	0.27	0.18	0.21	0.18	0.17
	80	0	0	0.03	0.01	0.02
	100	0.45	0.45	0.36	0.44	0.46
	110	0	0	0.03	0.02	0.02
	120	0	0	0.02	0	0.01
	140	0.18	0.24	0.19	0.26	0.19
	160	0.02	0.01	0.01	0	0.01
	180	0.06	0.07	0.06	0.08	0.08
	210	0.01	0	0.02	0.01	0
*LDH-1**	80	0.06	0.05	0.01	0.02	0.02
	100	0.84	0.89	0.91	0.96	0.93
	110	0.08	0.03	0.03	0	0.01
	180	0.02	0.04	0.05	0.02	0.04
*LDH-2**	100	0.88	0.92	0.91	0.94	0.90
	120	0.12	0.08	0.09	0.06	0.10
*ADA**	70	0.05	0.02	0.05	0.05	0.03
	80	0.20	0.10	0.30	0.13	0.19
	90	0.04	0.06	0.09	0.15	0.14
	100	0.42	0.48	0.30	0.36	0.38
	110	0.06	0.01	0.07	0.02	0.06
	120	0.12	0.26	0.14	0.23	0.14
	130	0.10	0.06	0.03	0.03	0.05
	140	0.01	0.01	0.03	0.03	0.01
*GDA**	70	0	0	0	0	0.01
	80	0.01	0.03	0.03	0.12	0.10
	90	0.21	0.25	0.22	0.14	0.22
	100	0.44	0.32	0.36	0.34	0.39
	110	0.15	0.22	0.15	0.16	0.10
	120	0.04	0.12	0.16	0.11	0.10
	130	0.15	0.05	0.07	0.11	0.07

Table 1. *Continued*

Locus	Allele	Águilas (N = 262)	Cape of Palos (N = 269)	Guardamar (N = 158)	Mazarrón (N = 267)	Tabarca (N = 293)
*IDHP-1**	60	0.19	0.21	0.18	0.27	0.20
	80	0.06	0.06	0.05	0.04	0.05
	100	0.55	0.55	0.55	0.53	0.55
	110	0.02	0.02	0.02	0.04	0.03
	120	0.15	0.14	0.16	0.08	0.15
	130	0.01	0.01	0	0.02	0
	140	0.02	0.02	0.04	0.01	0.02
*IDHP-2**	100	0.56	0.51	0.49	0.53	0.51
	120	0.44	0.49	0.51	0.47	0.49
*AAT-1**	80	0	0.01	0.06	0.02	0.01
	90	0.15	0.11	0.14	0.19	0.19
	100	0.73	0.78	0.69	0.70	0.71
	110	0.02	0.07	0.03	0.01	0.02
	120	0	0	0	0	0.01
	125	0.10	0.04	0.08	0.08	0.06
*AAT-2**	80	0.02	0.03	0.03	0.02	0.04
	90	0.22	0.22	0.20	0.23	0.21
	100	0.57	0.53	0.57	0.54	0.59
	110	0.14	0.13	0.15	0.15	0.12
	120	0.05	0.09	0.04	0.06	0.04
	120	0.04	0.09	0.08	0.06	0.11
*MDH-1**	40	0.01	0.01	0.01	0.01	0.03
	60	0.14	0.10	0.14	0.13	0.09
	80	0.34	0.28	0.26	0.27	0.28
	90	0	0.002	0	0	0
	100	0.48	0.52	0.52	0.52	0.48
	120	0.04	0.09	0.08	0.06	0.11
*MDH-2**	60	0.20	0.12	0.23	0.28	0.17
	100	0.57	0.61	0.55	0.53	0.51
	120	0.23	0.26	0.22	0.19	0.31

F-statistics following Wright (1951) were calculated to detect non-random mating within populations (F_{IS}) and differentiation between populations (F_{ST}). Both statistics were calculated by the method of Weir & Cockerham (1984) using their equations 1 and 4 for single locus values and equation 10 for multiple loci. Probabilities of random departure from zero for *F*-values, according to the null hypothesis, were read directly from the distribution of 1000 randomized matrices computed by permutation of individuals between populations. This was performed using the *F*-testing procedure of the 'Genetix' package, thus allowing a test of significance. Appropriate variances were calculated by jack-knife procedures found in the Genetix package and these were used to construct 95% confidence limits for each estimate.

Genetic distance (*D*; Nei, 1978) was computed between pairwise samples and the resulting matrix was clustered using Euclidean distance and UPGMA (unweighted pair-group method of arithmetic averages) algorithm (Statistica, v. 6.0). Probabilities of random departure from zero for Nei's *D*-values, according to the null hypothesis, were read directly from the distribution of 1000 randomized matrices computed by permutation (Genetix package).

Nei's *D*-values were associated with the geographical distances measured as the shortest marine route between the locations sampled. Also, correlation between geographical and genetic distances were tested using Mantel's test of the association between two parameters in data matrices with internal correlation (Mantel, 1967). Probabilities were read directly from the distribution of 1000 randomized matrices computed by permutation. Mantel's test was performed using the MANTEL procedure of the Genetix package.

Comparisons with the north-western Mediterranean were performed using data from Lenfant (1998). Only the allelic frequencies of polymorphic loci screened in both works (Lenfant, 1998 and González-

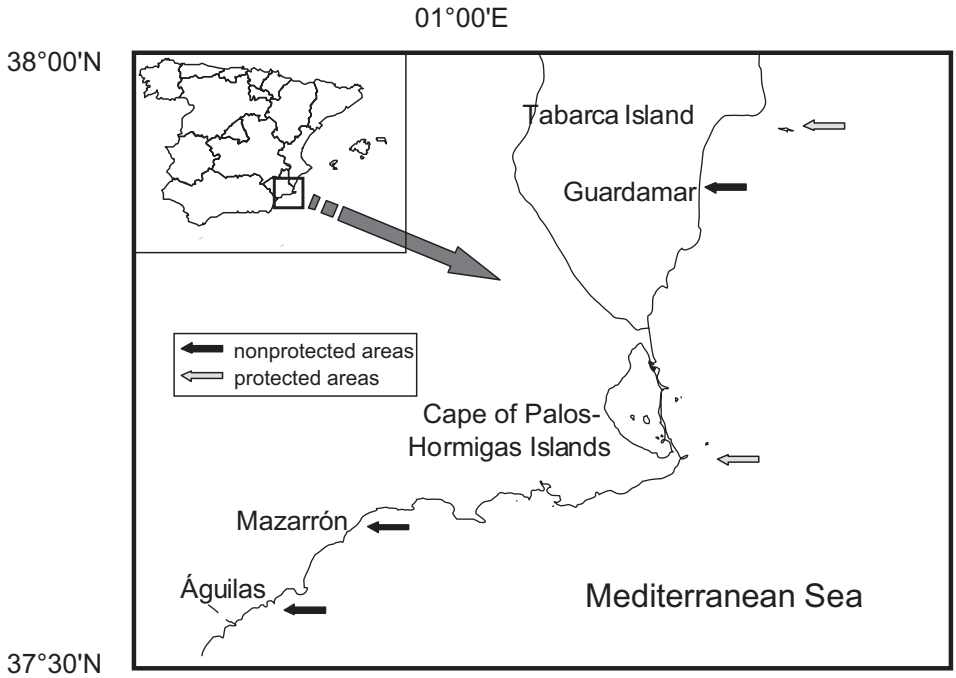

Figure 1. Study sampling sites for white sea bream *Diplodus sargus*.

Table 2. Description of genetic diversity in sampled populations of *Diplodus sargus*

Population	*N*	A_T	$P_{95\%}$	$P_{99\%}$	$H_o \pm SE$	$H_e \pm SE$
Águilas	262	5.07	1.00	1.00	0.3829 ± 0.1536	0.5696 ± 0.1600
Cape of Palos	269	5.00	1.00	1.00	0.4182 ± 0.2152	0.5499 ± 0.1841
Guardamar	158	5.36	1.00	1.00	0.3795 ± 0.1699	0.5779 ± 0.1966
Mazarrón	267	5.14	0.93	1.00	0.3569 ± 0.1752	0.5568 ± 0.2135
Tabarca	293	5.57	1.00	1.00	0.3138 ± 0.1632	0.5654 ± 0.1962

A_T: average number of alleles; $P_{95\%}$ and $P_{99\%}$: polymorphisms; H_o: observed heterozygosity; H_e: expected heterozygosity; SE: standard error).

Wangüemert *et al*., 2002) were used. A calibration was carried out at the University of Perpignan. Samples from both works were run side by side for all loci showing the same mobility at the same locus.

Data were analysed using the Genepop software (Raymond & Rousset, 1995a) and Genetix package (Bonhomme *et al*., 1993) (available at: http://www.University-montp2.fr/genome-pop/genetix.htm).

Genetic differences were also compared in a multivariate ordination, using factorial correspondence analysis on the allele frequencies. These analyses have been described by She *et al*. (1987) and were calculated using the CANOCO package.

Gene flow between samples was estimated as the number of migrants exchanged between populations per generation at equilibrium (N_em). Values for N_em were derived from one approach with F_{ST} values, following the island model of Wright (1951) with a small level of migration, where

$$N_em = (1 - F_{ST})/4\ F_{ST}$$

The private alleles method was also used (Barton & Slatkin, 1986).

RESULTS

Electrophoresis performed on nine enzymes gave a total of 14 polymorphic loci. Allelic frequencies are presented in Table 1. Populations from Tabarca and Cape of Palos marine reserves were characterized by unique alleles (*GPI-2**40, *AAT*-1*120 and *GDA**70, and *MDH-1**90, respectively).

All the loci were polymorphic ($P = 0.95$) (Table 2). The average number of alleles per population varied

from 5.00 in Cape of Palos to 5.57 in Tabarca. The mean observed heterozygosity (H_o) ranged from 0.3138 in Tabarca to 0.4182 in Cape of Palos.

Genic and genotypic differentiation (Table 3) was detected between the five sampled populations. Four loci showed significant differentiation over all population pairs (*GPI-1**, GPI-2*, PGDH* and *ADA**). Of the loci exhibiting significant differentiation, all showed a significant divergence form Hardy–Weinberg equilibrium (Table 4).

F_{IS} (Weir & Cockerham, 1984) values indicated a higher heterozygote deficit in Tabarca island ($F_{IS} = 0.45$) than in coastal areas, of which Cape of Palos showed the highest observed heterozygosity and the lowest deficit of heterozygotes ($F_{IS} = 0.24$). F_{IS} values per locus ranged from 0.0028 to 0.7941 (Table 4). The highest deficit of heterozygotes was shown by the *LDH-2* locus ($F_{IS} = 0.7941$) and no loci showed an excess of heterozygotes.

Nei's genetic distances were estimated using 14 polymorphic loci (Table 5). Values ranged from 0.006 to 0.013. All distances were significant at the 0.05 level. The lowest *D*-values were obtained among Guardamar and Tabarca populations, while the highest value of genetic distance ($D = 0.013$) was obtained between Guardamar and Cape of Palos. Estimates of genetic subdivision (F_{ST}) in the five samples are also given in Table 5.

F_{ST} (Weir & Cockerham, 1984) was 0.00650 with a 95% CI using bootstrapping over loci of 0.0033–0.0099. The minimum F_{ST} value derived from allelic variation observed was between Guardamar and Tabarca samples (0.004), indicative of the low divergence in gene frequencies between the two populations, and between Águilas and Mazarrón with Tabarca (0.005). In contrast, F_{ST} was considerably higher (0.01) between Cape of Palos and Guardamar samples and between Águilas and Mazarrón samples (0.008), suggesting the occurrence of restricted gene flow between these populations. F_{ST} values between samples were significant at level of 0.05 confirming the genetic differentiation between populations. Some allozyme loci such as *GDA**, ADA*, MDH-2* and *LDH-1** showed greater genetic differentiation from other loci and would be responsible for the F_{ST} differences.

The possibility of gene flow explaining allelic distribution was examined by comparing geographical and genetic distances (Fig. 2). The regression between corresponding values in the two matrices was low ($r^2 = 0.09$) although the same analysis excluding the Guardamar–Tabarca pair improved the regression value ($r^2 = 0.67$) and showed decreasing *D* with increasing geographical distance. For all samples combined, Pearson's coefficient ($r = -0.29$) was not significant as indicated by the Mantel test ($G = -0.93$, $P = 0.78$). For the studied samples excluding the Guar-

Table 3. Genotypic (G) and genic (g) differentiation at 14 loci for each population pair of *Diplodus sargus*

Population pair	GPI-1		GPI-2		PGM		PGDH		LDH-1		LDH-2		ADA		GDA		IDHP-1		IDHP-2		AAT-1		AAT-2		MDH-1		MDH-2	
	G	g	G	g	G	g	G	g	G	g	G	g	G	g	G	g	G	g	G	g	G	g	G	g	G	g	G	g
CP-A	**	**	***	***	NS	NS	***	***	**	***	NS	NS	***	***	***	***	NS	NS	NS	NS	***	***	NS	NS	*	**	**	**
G-A	***	***	***	***	NS	NS	***	***	***	***	NS	NS	***	***	***	***	NS	NS	*	NS	***	***	NS	NS	NS	NS	NS	NS
G-CP	***	***	***	***	NS	*	***	***	*	**	NS	NS	***	***	NS	NS	NS	NS	NS	NS	***	***	NS	NS	NS	NS	**	***
M-A	***	***	***	***	NS	NS	***	***	***	***	*	**	***	***	***	***	*	***	NS	NS	*	***	NS	NS	NS	NS	*	**
M-CP	***	***	***	***	*	NS	*	***	***	***	NS	NS	***	***	***	***	*	**	NS	NS	***	***	NS	NS	NS	NS	***	***
M-G	***	***	***	***	NS	**	***	***	***	***	NS	NS	***	***	***	***	***	***	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
T-A	**	***	***	***	NS	*	***	***	***	***	NS	NS	***	***	***	***	NS	NS	NS	NS	*	***	NS	NS	***	***	*	*
T-CP	**	***	***	***	*	NS	***	***	*	***	NS	NS	***	***	***	***	NS	NS	NS	NS	***	***	NS	**	NS	NS	**	NS
T-G	***	***	*	***	NS	*	*	**	NS	*	NS	NS	**	***	***	***	NS	NS	NS	NS	*	***	NS	NS	NS	NS	*	**
T-M	***	***	***	***	NS	NS	***	***	NS	*	NS	NS	***	***	***	***	**	***	NS	NS	NS	NS	*	*	*	**	***	***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. NS = not significant; A = Águilas; CP = Cape of Palos; G = Guardamar; M = Mazarrón; T = Tabarca; B = Banyuls; GI = Giglio; E = Elba; L = Livorno; MR = Marsella; BL = Blanes.

Table 4. F_{IS} values for each locus over all populations of *Diplodus sargus*

	Águilas		Cape of Palos		Guardamar		Mazarrón		Tabarca	
	F_{IS}	E.T/P	F_{IS}	E.T/P	F_{IS}	E.T/P	F_{IS}	E.T/P	F_{IS}	E.T/P
*GPI-1**	0.4473	***/*	0.3673	***/*	0. 1916	****/*	0.4340	****/*	0.4927	****/*
*GPI-2**	0.4310	***/*	0. 1850	***/*	0.3601	***/*	0.4631	***/*	0.5593	***/*
*PGM**	0.3751	***/*	0. 1856	***/*	0.3710	***/*	0.3134	***/*	0.3395	***/*
*PGDH**	0.3745	***/*	0.4357	***/*	0.3932	***/*	0.2742	***/*	0.4151	***/*
*LDH-1**	0.3030	***/*	0.5861	***/*	0.4099	***/*	0.4385	***/*	0.7239	***/*
*LDH-2**	0.3182	***/*	0.7941	***/*	0.2692	*/*	0.1066	*/NS	0.6583	***/*
*ADA**	0.3060	***/*	0.0571	***/NS	0.3662	***/*	0.3739	***/*	0.4696	***/*
*GDA**	0.1012	***/*	0.0105	***/NS	0. 1848	***/*	0. 1811	***/*	0.2253	***/*
*IDHP-1**	0.4277	***/*	0.3410	***/*	0.4282	***/*	0.5297	***/*	0.5873	***/*
*IDHP-2**	0.4973	***/*	0.4582	***/*	0.5590	***/*	0.5427	***/*	0.5694	***/*
*AAT-1**	0.6440	***/*	0.5585	***/*	0.7557	***/*	0.6741	***/*	0.7652	***/*
*AAT-2**	0. 1964	***/*	0. 1725	***/*	0.2917	***/*	0.2708	***/*	0.3194	***/*
*MDH-1**	0.1551	***/*	0.0650	***/NS	0.2421	***/*	0.2656	***/*	0.3540	***/*
*MDH-2**	0.1659	***/*	0.0028	***/NS	0.1533	*/NS	0.2252	***/*	0.3031	***/*
Multilocus	0.3294	*/*	0.2411	*/*	0.3461	*/*	0.3606	*/*	0.4464	*/*

Significance was tested according to the exact test (E.T) (Raymond & Rousset, 1995b) and permutations (P). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. NS = not significant.

Table 5. Pairwise Nei's genetic distances (D, below diagonal) and F_{ST} values (above diagonal) in *Diplodus sargus*

Locations	Águilas	Cape of Palos	Guardamar	Mazarrón	Tabarca
Águilas	–	0.007*	0.006*	0.008*	0.005*
Cape of Palos	0.009*	–	0.010*	0.007*	0.007*
Guardamar	0.009*	0.013*	–	0.008*	0.004*
Mazarrón	0.012*	0.010*	0.011*	–	0.005*
Tabarca	0.007*	0.010*	0.006*	0.008*	–

F_{ST} and Nei's D considered to be significantly different from zero (*) if they fall within the 5% most extreme values in the permutation test.

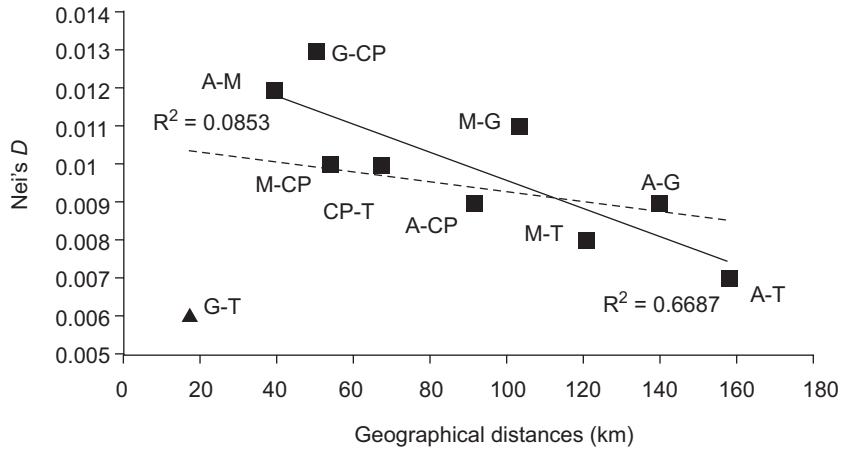

Figure 2. Relationship between genetic and geographical distances in *Diplodus sargus* populations using Nei's (1978) genetic distance, D. A = Águilas; CP = Cape of Palos; G = Guardamar; M = Mazarrón; T = Tabarca; continuous line = regression without G-T; discontinuous line = regression with G-T.

Table 6. Estimates of N_em using F_{ST} values (above diagonal) and private alleles (below diagonal) in *Diplodus sargus*

	Águilas	Cape of Palos	Guardamar	Mazarrón	Tabarca
Águilas	–	38.37	43.36	30.26	52.34
Cape of Palos	*	–	24.80	33.89	35.99
Guardamar	*	*	–	31.23	69.91
Mazarrón	*	4.46	*	–	47.90
Tabarca	*	1.26	*	2.12	–

*There are no private alleles between these population pairs.

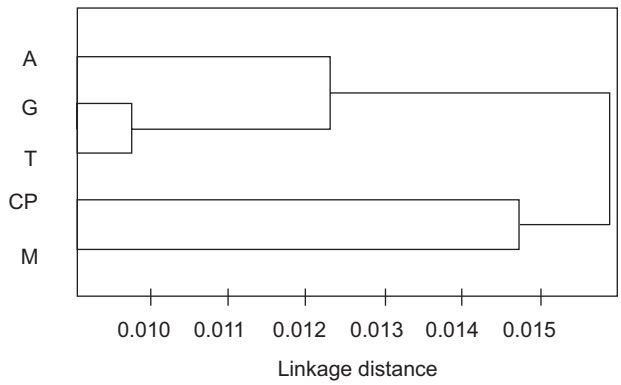

Figure 3. UPGMA cluster of genetic distances (Nei, 1978) among the five populations of *Diplodus sargus* sampled. A = Águilas; CP = Cape of Palos; G = Guardamar; M = Mazarrón; T = Tabarca.

damar sample, the *r*-value increased ($r = -0.96$) but neither was significant.

Assuming equilibrium between genetic drift and migration, we calculated from the F_{ST} values and according to the island model the number of migrants (N_em) per generation (Table 6). Estimates of the number of migrants exchanged between populations per generation were greater than 1: $N_em = 24.8$ (using F_{ST} values) and $N_em = 1.26$ (using private alleles).

The dendrogram performed using genetic distances (Nei, 1978; Table 5) reflected the geographical relationships among populations (Fig. 3). Guardamar and Tabarca populations clustered together, and the Águilas population joined this group; Mazarrón and Cape of Palos populations formed a distinct group in the dendrogram.

DISCUSSION

Allelic richness, the total number of alleles per population including all the loci considered, of the five populations analysed in the south-east of Spain was higher than observed values in the north-western Mediterranean populations considering the same loci (Lenfant, 1998), with the exception of the Banyuls population which has similar values to those of the south-east of Spain.

The studied populations were characterized by high levels of heterozygosity. The average heterozygosity estimated for the common loci in the five populations of *D. sargus* from the south-western Mediterranean (average $H_o = 0.4333$) was significantly higher ($P < 0.005$) compared with north-western Mediterranean populations (average $H_o = 0.3465$). Both are higher than values obtained for *D. sargus* populations from Brindisi in the southern Adriatic, Italy ($H_o = 0.1925$) (Cervelli, 1999) and than values for the observed heterozygosity in other coastal fish species: *Mullus barbatus,* which ranges from 0.077 in Greece to 0.164 in France (Mamuris *et al*., 1998), and *Mugil cephalus*, whose average heterozygosity was estimated at 0.050 (Rossi *et al*., 1998).

Organisms such as marine fishes, having large populations, would have larger heterozygosity values compared with organisms with smaller populations (Gyllensten, 1985). The large value of heterozygosity, therefore, could imply that *D. sargus* has probably had a long, unbroken history in the south-western Mediterranean Sea, perhaps without population bottlenecks (Mamuris *et al*., 1998).

However, despite their high heterozygosity, the five populations from the south-western Mediterranean also presented a deficit of heterozygotes. Genotypic proportions differed significantly from Hardy–Weinberg equilibrium in all populations although this tendency was more marked at Tabarca island. Apart from chance alone, a number of factors may be responsible for this deficit. These include inbreeding, null alleles, the Wahlund effect and selection against heterozygotes or strong directional selection as a consequence of the geographical isolation of some populations (Zouros & Foltz, 1984; Mamuris *et al*., 1998; Rossi *et al*., 1998).

Fishing can reduce genetic heterozygosity (Bergh & Getz, 1989) and lead to potentially adverse genetic selection on average size, growth rates, maturity and behaviour (Wilson & Clarke, 1996). However, the lower observed heterozygosity and higher deficit of heterozygotes occurred in populations in a protected area, Tabarca marine reserve (a stable environment

with low fishery pressure), which had the highest homozygosity and the highest allelic richness. The fact that this locality showed high rates of interchange of individuals would suggest that the observed deficit of heterozygotes could be due to the Wahlund effect.

The dendrogram of Nei's genetic distances illustrated the relatedness of the different populations. The calculation of *D* is based upon comparisons of allele frequencies and is not strongly affected by the presence of unique alleles at low frequencies in particular populations (Avise, 1994). The distinction of Mazarrón and Cape of Palos localities from the others is attributable to differences in the more common allele frequencies. The importance of both historical biogeography and contemporary gene flow in shaping intraspecific population genetic structure should be considered (Planes, 1992; Planes, Parroni & Chauvet, 1998).

The genetic consequences of larval dispersal are characterized by the balance between the forces of gene flow (which tend to make gene frequencies uniform among populations) and those of genetic drift and natural selection (which act to diversify populations) (Wright, 1931; Planes, 1993). The analysis could not discern whether selection or genetic drift is the main force causing the observed differences.

F_{ST} values were always lower than 0.1, indicating that there is little divergence among populations (Hartl, 2000). Tabarca showed the lowest values with all the other localities (F_{ST} = 0.005) except Cape of Palos, which showed the highest degree of isolation.

These results and the graphic representation of Nei's distance and genetic flow (Fig. 4) suggest that genetic flow takes place through the pelagic systems and open sea circulation model, which connect localities at the south of Cape of Palos (Águilas and Mazarrón) with the northern areas (Guardamar and Tabarca). However, communication between populations is very restricted through the coastal area at the south of Guardamar. Genetic fluxes are very low between Águilas, Mazarrón and Cape of Palos and this last locality shows in general the lowest genetic fluxes of all the areas.

The sampled populations of *D. sargus* had new alleles, which had not previously been detected in northwest Mediterranean populations (Lenfant & Planes, 1996a) (Table 7). These alleles may have arisen from Atlantic stocks given the present flow of adults or larval fish between both seas. Several authors (Bouchet & Taviani, 1992; Quesada, Zapata & Álvarez, 1995; Bembo *et al*., 1996) have demonstrated the passive dispersal of pelagic larvae of marine species through the Strait of Gibraltar. Further genetic studies using Atlantic samples are required to test this hypothesis.

Roldán *et al*. (1998) studied some hake populations from Atlantic and Mediterranean basins and concluded that gene exchanges were from the Atlantic to

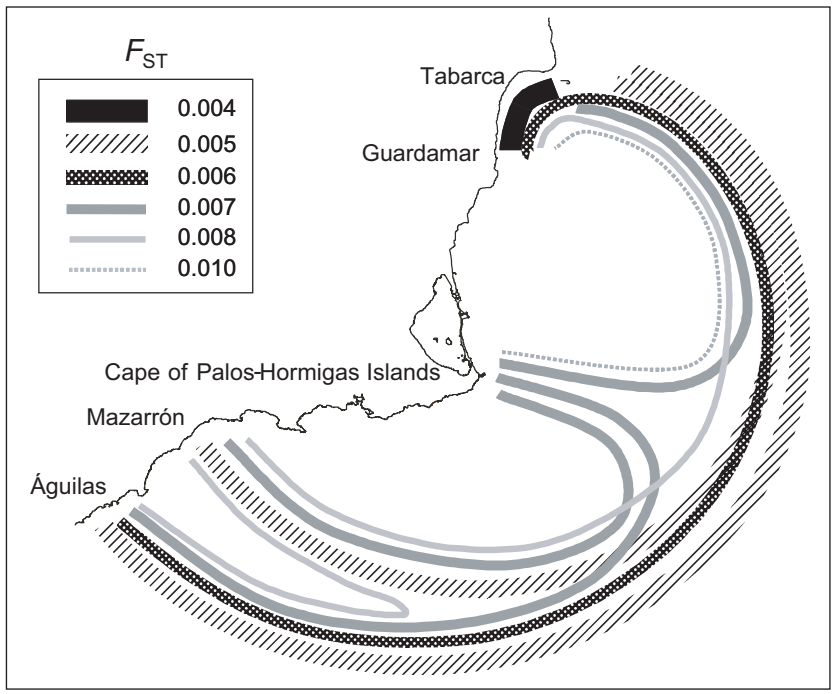

Figure 4. Map illustrating the patterns of genetic connectivity (F_{ST} values) inferred from the surveys of *Diplodus sargus*. Width of lines is proportional to gene flow. Data suggest that interchanges of individuals takes place through open sea currents and not through coastal areas.

Table 7. Allelic frequencies of alleles not shared between southern and northern Mediterranean populations of *Diplodus sargus*

	South-west Mediterranean					North-west Mediterranean					
Allele	A (N = 262)	CP (N = 269)	G (N = 158)	M (N = 267)	T (N = 292)	B (N = 215)	GI (N = 200)	E (N = 31)	L (N = 39)	MR (N = 50)	BL (N = 20)
*GPI2-40**	0	0	0	0	0.0037	0	0	0	0	0	0
*GPI2-60**	0.0625	0	0.1026	0.0061	0.0788	0	0	0	0	0	0
*GPI2-70**	0	0	0	0	0	0.029	0	0	0.0100	0	0.0057
*GPI2-85**	0	0	0	0	0	0.0126	0	0	0	0	0
*GPI2-90**	0	0	0	0	0	0.0417	0.0400	0.0256	0.0200	0.0250	0.0460
*GPI2-93**	0.0141	0	0.0298	0.0205	0.0293	0	0	0	0	0	0
*GPI2-105**	0	0	0	0	0	0.0006	0	0	0	0	0
*GPI2-110**	0	0	0	0	0	0.0023	0.0100	0	0	0	0.0057
*GPI2-140**	0	0.0060	0.0497	0	0.0751	0	0	0	0	0	0
*GPI2-167**	0	0.0536	0.0265	0.0164	0.0128	0	0	0	0	0	0
*GPI2-180**	0.0282	0.0020	0.0066	0	0.0128	0	0	0	0	0	0
*PGM-140**	0	0	0	0	0	0.0023	0	0.0128	0	0	0
*PGDH-40**	0.0172	0.0428	0.0823	0.0075	0.0377	0	0	0	0	0	0
*PGDH-70**	0	0	0	0	0	0.0709	0.1200	0.0789	0.0800	0.1000	0.0523
*PGDH-90**	0	0	0	0	0	0.0971	0.1000	0.1447	0.0900	0. 1750	0. 1724
*PGDH-140**	0. 1775	0.2416	0. 1899	0.2584	0. 1935	0	0	0	0	0	0
*PGDH-160**	0.0153	0.0149	0.0095	0.0019	0.0068	0	0	0	0	0	0
*PGDH-180**	0.0649	0.0706	0.057	0.0787	0.0771	0	0	0	0	0	0
*PGDH-210**	0.0134	0	0.0158	0.0094	0	0	0	0	0	0	0
*IDHP1-130**	0.0115	0.0075	0	0.0245	0.0035	0	0	0	0	0	0
*AAT1-95**	0	0	0	0	0	0.0006	0	0	0	0	0
*AAT1-125**	0.0992	0.0353	0.0759	0.0768	0.0634	0	0	0	0	0	0
*AAT2-115**	0	0	0	0	0	0	0	0	0	0	0.0057
*ADA-50**	0	0	0	0	0	0.0006	0	0	0	0	0
*MDH1-40**	0.0076	0.0149	0.0127	0.0131	0.0309	0	0	0	0	0	0
*MDH1-90**	0	0.0019	0	0	0	0	0	0	0	0	0

A = Águilas; CP = Cape of Palos; G = Guardamar; M = Mazarrón; T = Tabarca; B = Banyuls; GI = Giglio; E = Elba; L = Livorno; MR = Marsella; BL = Blanes.

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the Mediterranean. They also found a small genetic distance (D = 0.0188) between Murcia (Spanish Mediterranean) and Larache (Atlantic). Also, the genetic distance between Murcia and Peñones (North Africa, near Strait of Gibraltar) was lower than was the genetic distance between Murcia and Málaga (also Spanish Mediterranean).

A new dendrogram based on Nei's (1978) genetic distance was obtained from the data of common enzymes among north-western (Lenfant, 1998) and south-western Mediterranean populations (Fig. 5). Within geographical region, genetic distances segregated northern and southern populations of *D. sargus*. Figure 6 represents the multivariate space containing the first two axes of the factorial correspondence analysis, which together accounted for 91.43% the total variation. This analysis corroborates the conclusion from the cluster analysis, namely a major subdivision of samples from south-west Mediterranean from north-west Mediterranean samples along Axis I, which is most influenced by alleles that are not shared by north and south-west Mediterranean populations (Table 7).

Electrophoretic data in this study have shown that *D. sargus* populations on the scale of the western Mediterranean are not genetically homogeneous at spatial scales of from 10^2 to 10^3 km. The appearance of new

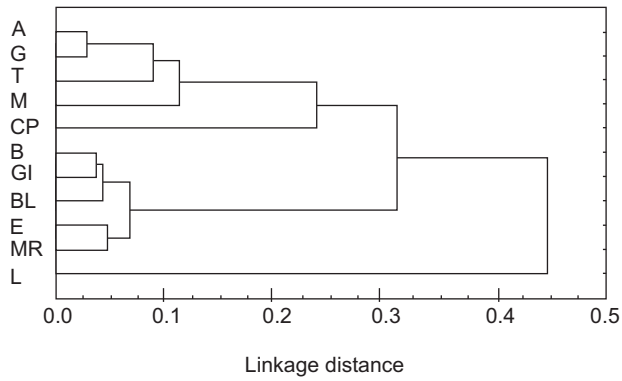

Figure 5. UPGMA cluster of genetic distances (Nei, 1978) among populations in the western Mediterranean. A = Águilas; CP = Cape of Palos; G = Guardamar; M = Mazarrón; T = Tabarca; B = Banyuls; GI = Giglio; E = Elba; L = Livorno; MR = Marsella; BL = Blanes.

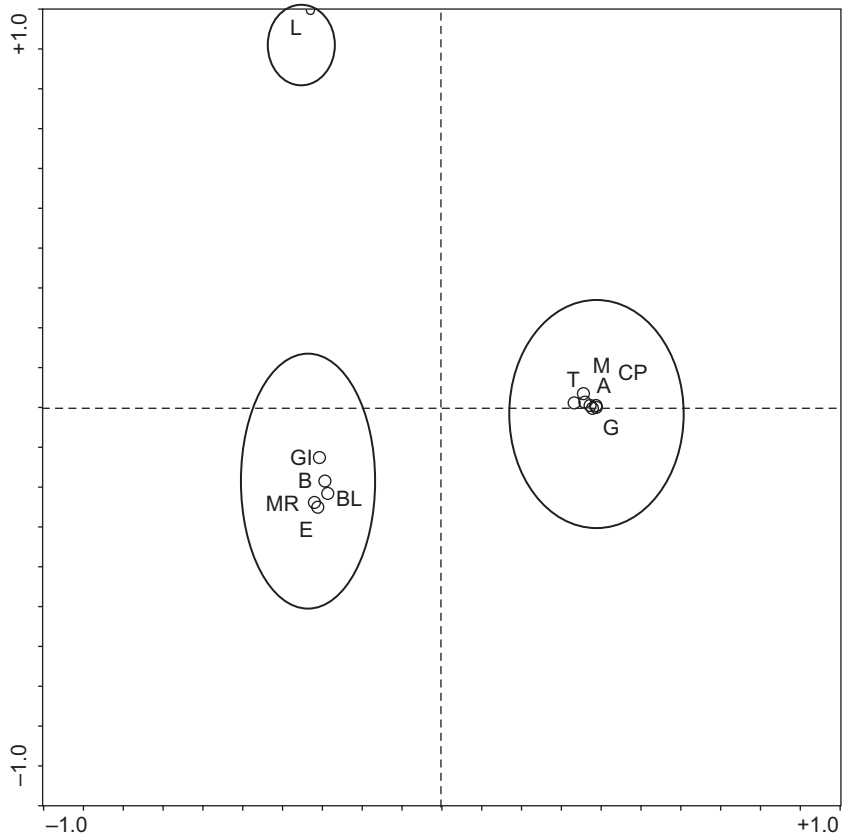

Figure 6. Scaleless ordination of first two axes of principal component analysis in *Diplodus sargus* that jointly explained 94% of the variance in the global data set. The 11 populations are plotted in the same multivariate space. Ellipses contain samples from different geographical regions: south-west and north-west Mediterranean. A = Águilas; CP = Cape of Palos; G = Guardamar; M = Mazarrón; T = Tabarca; B = Banyuls; GI = Giglio; E = Elba; L = Livorno; MR = Marsella; BL = Blanes.

alleles in the south-western Mediterranean populations of *D. sargus* could indicate the input of alleles from an Atlantic gene pool. The absence of work on this topic in populations from the north of Africa precludes one from having any idea of the degree of genetic homogeneity in this region.

According to these results any attempt to manage and protect the genetic diversity of *D. sargus* should not be made at a local or regional scale but at the scale of the Mediterranean Sea.

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