Genetic variation in natural stocks of *Sardina pilchardus* (sardines) from the western Mediterranean Sea

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Eleven enzymes coded by 15 loci (*ADH**, *SOD**, *SDH**, *LDH-1**, *LDH-2**, *ME-1**, *ME-2**, *GPI-1**, *GPI-2**, *IDH**, *MDH-1**, *MDH-2**, *PGM**, *AAT** and *CK**) were studied using electrophoretic methods in order to characterize stocks of sardines from the western Mediterranean Sea. The results showed differentiated groups distributed throughout the area studied. They did not form a panmictic population, but they existed as semi-independent, although not completely isolated, breeding units with an estimation of migration of $N_e m = 3.1$ (number of migrants per generation). In the LDH-1 system a north–south cline was detected. We have also detected a discontinuity between the Alboran and the rest of the Mediterranean populations sampled at the enzymatic level. This result confirms the action of the Almeria–Oran front as a barrier that produces discontinuities as detected in other marine species.

Keywords: genetic variation, isozyme polymorphism, sardine, fish stocks.

Introduction

The use of biological resources requires a knowledge of genetic variability within species. Few species form single homogeneous populations, and fish species are often composed of discrete stocks so that the relationship between them may be independent to a certain degree. The knowledge of the structure of each stock is important for a better commercial exploitation (Carvalho & Hauser, 1994).

A central problem in studies of fish populations is the definition of the concept of stock, which can be defined in different ways. From a genetic point of view, a stock is a reproductively isolated unit, which is genetically different from other stocks (Jamieson, 1973; Gyllensten, 1985; Ward *et al.*, 1994). The stock unit actually applied in management decisions depends in part on the amount of population data available to assess each stock separately.

Diverse characteristics and methods have been used to analyse stock structure. Allozyme polymorphisms have proven valuable for estimating population divergence and identifying discrete fish stocks. Their utility depends very much on the level of detectable genetic variation (Shaklee, 1983). In

several species with little genetic variability (for example squid, Atlantic salmon, sockeye salmon and many lobsters and prawns) biochemical studies need extremely large sample sizes and the dependence of the analysis on a few polymorphic gene loci has been severely criticized by some authors (Shaklee, 1983; Smith *et al.*, 1990). Nevertheless, some species show high genetic variation and in this case, the allozyme polymorphisms have been widely applied (Altukhov & Salmenkova, 1987; Shaklee *et al.*, 1990; Skaala *et al.*, 1990; Utter, 1991).

Marine fish species show lower levels of genetic differentiation than freshwater or anadromous species, probably owing to less pronounced barriers to migration and gene flow (Gyllensten, 1985; Ward *et al.*, 1994). However, in some cases we can find examples of barriers that restrict migration among stocks to a certain degree. One of these examples is in the Mediterranean Sea. South-eastern Spain is an area widely recognized as a biogeographical border between Mediterranean and Atlantic biotic communities (Rodriguez *et al.*, 1979). The existence of an Almeria–Oran oceanographic front, a well-defined hydrographic boundary between Atlantic and Mediterranean surface waters (Tintoré *et al.*, 1988), is the factor responsible for the current position and maintenance of discontinuities at the allozyme level in

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mussels (Quesada *et al.*, 1995a), oysters (Saavedra *et al.*, 1993) and at the mtDNA level in mussels (Quesada *et al.*, 1995b).

The purpose of this study was the characterization of genetic variation, at the enzymatic level, of the populations of sardines from the western Mediterranean Sea and to reveal the existence of a genetic discontinuity at the allozymic level in the Almeria–Oran front as found in other marine species.

Materials and methods

Collection of samples

Samples of *Sardina pilchardus* were collected by the Instituto Español de Oceanografía (ECOMED90 project, summer of 1990) on the continental platform along the Spanish Mediterranean coast as depicted in Fig. 1. The geographical location and the mean number of fishes (sample size) per locus for each population is indicated in Table 1.

Fish were collected and their heart, liver and ovaries or testes were extracted and immediately frozen on board the cruise ship. Upon arrival at the laboratory they were frozen to −80°C at which they remained until processed by electrophoresis.

Assays

Samples were analysed by horizontal starch gel electrophoresis. Information about the loci was extracted from the specific literature for fish, and the loci were scored using the same tissue source

Fig. 1 Samples of *Sardina pilchardus* collected along the Spanish Mediterranean coast. See Table 1 for an exact geographical location of the captures.

(mixture of liver, heart and ovaries and/or testes) and buffer systems. For the study of ADH (alcohol dehydrogenase, EC 1.1.1.1), SOD (superoxide dismutase, EC 1.15.1.1), SDH (sorbitol dehydrogenase, EC 1.1.1.14), LDH (lactate dehydrogenase, EC 1.1.1.27), PGI (glucose-6-phosphate isomerase, EC 5.3.1.9), PGM (phosphoglucomutase, EC 5.4.2.2), and CK (creatine kinase, EC 2.7.3.2) buffers were prepared and samples electrophoresed following the methods described by Ridgway *et al.* (1970). AAT (aspartate transaminase, EC 2.6.1.1) and MDH (malate dehydrogenase, EC 1.1.1.37) buffers were prepared following the methods of Clayton & Tetriak (1972), and those for IDH (isocitrate dehydrogenase, EC 1.1.1.42) and ME (malic enzyme, EC 1.1.1.40) using the methods of Wolf *et al.* (1970). Specific staining for each enzyme was performed following methods outlined in Harris & Hopkinson (1976) and Allendorf *et al.* (1977).

From the electrophoretic data, allele and genotype frequencies were estimated, and the Hardy–Weinberg equilibria were tested by the BIOSYS-1 program (Swofford & Selander, 1989). The Bonferroni procedure was used to correct the deviations in the χ^2-test which originated from the number of tests performed (Weir, 1990). The number of alleles per locus, the observed heterozygosity and the proportion of polymorphic loci were calculated for each system and sample.

Patterns of geographical variation were examined in order to detect the existence of clinal variation by means of the Pearson correlation coefficient for arcsine-transformed allele frequencies with geographical distances. To facilitate the comparison between alleles at different frequencies, these were transformed to standardized deviates and expressed in standard deviation units (Quesada *et al.*, 1995a).

The average heterozygosity was evaluated and the data were analysed for heterogeneity by means of F-statistics (F_{IS}, F_{IT}, F_{ST}) (Wright, 1951). Nei's genetic distance (Nei, 1972) was employed for the construction of dendrograms. All these data were calculated with the BIOSYS-1 program (Swofford & Selander, 1989). The significance of the cluster was tested by means of the bootstrap method, using the programs SEQBOOT, GENDIST, NEIGHBOR and CONSENSE, of the PHYLIP package (Felsenstein, 1993).

Results

Table 1 shows the parameters of genetic variability in the samples of *Sardina*. As can be seen, for the proportion of polymorphic loci the range was from
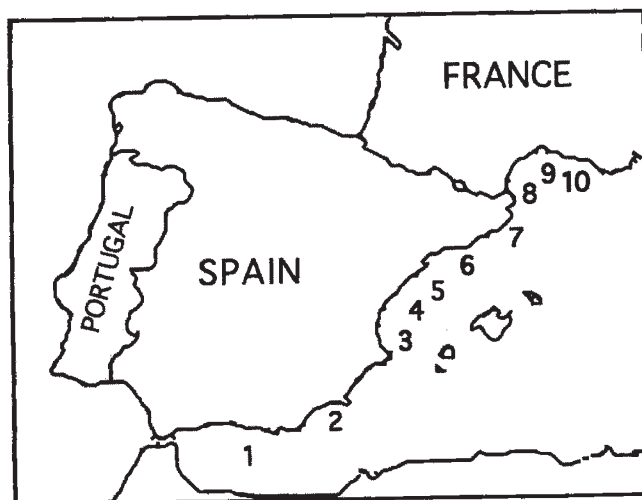
0.467 (Alicante) to 0.733 (Valencia-1 and Gulf of Lyon-3). With respect to heterozygosity, the range was from 0.110 (Valencia-1) to 0.160 (Gulf of Lyon-1); the number of effective alleles per locus was around 2 in all samples. The allele frequencies observed in the 10 samples for the 15 enzymatic loci are given in Table 2; the loci, except $LDH-2*$, were polymorphic in some of the samples.

The structure of genetic variation in the sardine samples was evaluated by means of F-statistics. Table 3 presents the results of the analysis; the F_{IS} for the loci show negative values for some of them but they are not statistically significant. The significant values were always positive indicating a lack of equilibrium caused by a deficiency of heterozygotes. The F_{IS} and F_{IT} globals (for the loci and the average) displayed positive values and were statistically significant in all systems, with the exception of $LDH-2*$, thus indicating a deficiency in the number of observed heterozygotes in the samples with respect to the expected number. F_{ST} was significant for every locus, except $LDH-2*$, and on average. These values indicate that the sardine population in the western Mediterranean is globally structured into different subpopulations, which are genetically differentiated. However, we have to ask if the genetical differentiation was the same for all the populations. To answer this, the F_{ST} between paired populations was estimated, and the results are in Table 4. These F_{ST} values were significant, with some exceptions; but the main thing is that Alboran showed, in general, the highest F_{ST} compared to the

rest of the populations, which clearly indicates the genetical differentiation of this population in relation to the others. To confirm this, a Student's t-test between the mean of the F_{ST} values of Alboran with the other nine populations (column 1 of Table 4) and the mean of the remaining F_{ST} values was made, and the analysis gave a $t_8 = 4.1$ $P < 0.005$), clearly significant. It is important to note that the three samples from the Gulf of Lyon are homogeneous.

The genetic differentiation between the subpopulations was also estimated by means of Nei's genetic distance. The UPGMA dendrogram (Fig. 2a) with all loci and populations sampled, showed one cluster containing the populations of Catalonia and Valencia with the exception of Valencia-3, another cluster with the three Gulf of Lyon populations, and the Alicante and Valencia-3 populations joined to them. The Alboran population with 0.027 units of distance made up the third cluster. The circled numbers are bootstrap per cent values based on 1000 pseudo-repetitions. All the bootstrap values were very low, with the exception of Alboran, with 51 per cent. This value, although low, indicated the genetic separation of this population with respect to the others, which together form a single big cluster.

Based on the pattern and magnitude of the allelic frequency variation among the populations, the loci analysed could be classified into two groups. One of them included five loci ($SOD*$, $MDH-2*$, $PGM*$, $AAT*$ and $CK*$) which exhibited the highest variation of frequency based on standard deviation units (mean $SDU = 0.115$), and the second group was

Table 1 Collection localities and estimates of genetic variability (with standard errors) in samples of *Sardina pilchardus* from the western Mediterranean Sea. The number of loci scored in all samples was 15 from 11 enzyme systems (see text for details)

Sample	Geographical location	Mean sample size per locus	Mean no. of alleles per locus	Proportion of loci	
				Polymorphic* per sample	Heterozygous per sample
1 Alboran	36°33′N, 03°10′W	41.9 ± 2.0	1.7 ± 0.2	0.533	0.114 ± 0.031
2 Alicante	37°32′N, 01°17′W	34.9 ± 2.1	1.7 ± 0.1	0.467	0.124 ± 0.036
3 Valencia-1	39°12′N, 00°05′E	41.2 ± 1.9	2.1 ± 0.1	0.733	0.110 ± 0.024
4 Valencia-2	39°57′N, 00°22′E	42.5 ± 2.6	2.0 ± 0.2	0.533	0.128 ± 0.032
5 Valencia-3	40°23′N, 00°55′E	44.9 ± 1.5	1.8 ± 0.1	0.667	0.133 ± 0.027
6 Catalonia-1	40°54′N, 01°00′E	44.7 ± 2.5	1.9 ± 0.1	0.600	0.112 ± 0.020
7 Catalonia-2	41°31′N, 02°37′E	68.4 ± 3.6	2.1 ± 0.2	0.533	0.140 ± 0.035
8 Gulf of Lyon-1	42°59′N, 03°38′E	45.2 ± 2.1	1.7 ± 0.1	0.667	0.160 ± 0.034
9 Gulf of Lyon-2	43°13′N, 04°21′E	45.0 ± 1.7	2.0 ± 0.1	0.600	0.148 ± 0.036
10 Gulf of Lyon-3	43°16′N, 04°37′E	46.7 ± 1.7	2.1 ± 0.1	0.733	0.133 ± 0.020

*A locus is considered polymorphic when the frequency of the most common allele does not exceed 0.95.

Table 2 Allele frequencies for 15 isozyme loci in 10 samples of *Sardina pilchardus* from the western Mediterranean Sea. Sample locations are described in Table 1

Locus	Allele	1	2	3	4	5	6	7	8	9	10
						Sample					
*ADH**	N	47	37	36	18	47	37	76	47	48	49
	90	0.032	0.122	0.069	0	0.117	0.027	0.033	0.128	0.167	0.122
	100	0.968	0.878	0.931	1	0.883	0.973	0.967	0.872	0.833	0.878
*SOD**	N	38	34	50	49	48	48	75	49	48	50
	100	0.263	0.221	0.090	0.071	0.083	0.146	0.073	0.214	0.115	0.060
	130	0.737	0.779	0.910	0.929	0.917	0.854	0.927	0.786	0.885	0.940
*SDH**	N	48	20	49	31	29	12	31	25	28	27
	90	0.010	0.050	0.051	0.226	0.328	0.042	0	0.100	0.125	0.037
	100	0.990	0.950	0.949	0.774	0.672	0.958	1	0.900	0.875	0.963
*LDH-1**	N	46	50	48	50	48	48	76	50	50	50
	80	0	0	0.010	0	0.156	0.073	0.020	0.230	0.100	0.150
	100	1	1	0.990	1	0.844	0.927	0.980	0.770	0.900	0.850
*LDH-2**	N	46	50	48	50	48	48	76	50	50	50
	90	0	0	0	0	0	0.031	0.020	0	0	0.020
	100	1	1	0.990	1	1	0.969	0.980	1	1	0.980
	120	0	0	0.010	0	0	0	0	0	0	0
*ME-1**	N	48	33	35	50	48	49	74	50	44	50
	90	0.146	0	0.043	0.090	0.198	0.122	0.081	0	0.045	0.120
	100	0.854	1	0.957	0.910	0.802	0.878	0.919	1	0.955	0.880
*ME-2**	N	48	36	35	50	48	49	74	50	44	50
	95	0.094	0.097	0.071	0.040	0	0	0.007	0.190	0.102	0.070
	100	0.906	0.903	0.929	0.960	1	1	0.993	0.810	0.898	0.930
*PGI-1**	N	42	37	46	50	40	47	73	50	48	50
	100	0.929	0.973	0.826	0.970	0.863	0.851	0.774	0.920	0.854	0.910
	140	0.071	0.027	0.174	0.030	0.137	0.149	0.226	0.080	0.146	0.090
*PGI-2**	N	42	37	46	50	41	47	73	50	50	50
	100	1	0.986	0.924	0.970	1	0.968	1	1	0.990	0.970
	130	0	0.014	0.076	0.030	0	0.032	0	0	0.010	0.030
*IDH**	N	48	20	36	50	50	50	74	50	50	50
	80	0	0	0	0	0	0	0.006	0	0	0.010
	90	0	0	0.028	0.110	0.010	0.230	0.223	0.020	0.020	0.010
	100	1	0.975	0.972	0.880	0.990	0.770	0.757	0.980	0.970	0.940
	120	0	0.025	0	0.010	0	0	0.014	0	0.010	0.040
*MDH-1**	N	30	34	33	33	41	50	72	46	46	50
	90	0	0.015	0.076	0.030	0.098	0.150	0.125	0.152	0.033	0
	100	0.917	0.985	0.924	0.970	0.793	0.850	0.875	0.848	0.967	0.910
	120	0.083	0	0	0	0.109	0	0	0	0	0.090
*MDH-2**	N	27	33	33	33	41	45	72	47	46	47
	80	0.093	0	0.091	0.061	0	0.133	0	0	0	0
	100	0.722	1	0.879	0.939	1	0.867	0.972	1	1	0.968
	110	0	0	0	0	0	0	0.028	0	0	0.032
	null	0.185	0	0.030	0	0	0	0	0	0	0
*PGM**	N	46	29	30	47	45	43	66	46	45	50
	100	1	0.638	0.550	0.500	0.889	0.558	0.803	0.804	0.778	0.860
	140	0	0.362	0.450	0.500	0.111	0.442	0.197	0.196	0.222	0.140
*AAT**	N	28	37	47	37	51	50	75	40	31	38
	50	0	0	0	0.014	0	0	0.007	0	0.016	0
	75	0.375	0.230	0.319	0.108	0.167	0.040	0.280	0.200	0.194	0.224
	100	0.625	0.770	0.681	0.878	0.833	0.960	0.713	0.800	0.790	0.776
*CK**	N	44	36	46	40	49	47	39	28	47	39
	85	0.614	0.528	0.196	0.500	0.296	0.245	0.308	0.464	0.277	0.526
	100	0.386	0.472	0.804	0.488	0.704	0.755	0.692	0.536	0.723	0.474
	120	0	0	0	0.012	0	0	0	0	0	0

N, number of individuals.

Table 3 F-statistics for each locus in the western Mediterranean populations of *Sardina pilchardus*. F_{IS} is calculated for each locus in each population

Population	ADH*	SOD*	SDH*	LDH-1*	LDH-2*	ME-1*	ME-2*	PGI-1*	PGI-2*	IDH*	MDH-1*	MDH-2*	PGM*	AAT*	CK*	Average
1	-0.033	0.457*	-0.011	—	—	0.164	-0.103	-0.077	—	—	0.345	0.745**	—	0.162	0.425*	
2	0.115	0.401	-0.053	—	—	—	0.209	-0.028	-0.014	-0.026	-0.015	—	0.179	0.160	0.220	
3	0.355	0.145	-0.054	-0.011	-0.011	-0.045	0.785**	0.546**	0.536**	-0.029	-0.082	1.000**	0.394	0.315	0.447*	
4	—	-0.077	0.262	—	—	0.145	-0.042	0.656**	0.656**	0.250	-0.031	-0.065	0.191	0.126	0.365	
5	0.279	0.455*	0.609*	0.131	—	0.409**	—	0.051	—	-0.010	0.234	—	-0.125	0.224	0.461*	
6	-0.028	0.666**	-0.043	0.230	-0.032	0.620**	—	0.664**	-0.033	0.266	0.294	0.423*	0.717**	-0.042	0.252	
7	0.380**	-0.079	—	-0.020	-0.020	0.093	-0.007	0.491**	—	0.212	0.111	-0.029	0.138	0.451**	-0.083	
8	0.045	-0.030	0.333	0.266	—	—	0.415*	0.185	—	-0.020	-0.011	—	-0.105	0.062	0.569*	
9	0.250	0.897**	0.510	-0.111	—	-0.048	-0.114	0.498**	-0.010	-0.024	-0.034	—	-0.157	-0.242	0.256	
10	0.430*	0.645***	-0.038	0.451*	-0.020	0.242	-0.075	-0.099	-0.031	-0.047	0.145	-0.033	0.169	0.470*	0.332	
F_{IS}†	0.222	0.360	0.331	0.203	-0.024	0.261	0.182	0.347	0.299	0.170	0.141	0.565	0.208	0.198	0.327	0.267
F_{IT}†	0.252	0.387	0.407	0.274	-0.007	0.296	0.223	0.372	0.318	0.257	0.183	0.610	0.311	0.238	0.381	0.321
F_{ST}	0.038**	0.042**	0.113**	0.089**	0.016	0.048**	0.050**	0.037**	0.028*	0.105**	0.049**	0.103**	0.130**	0.050**	0.081**	0.074**
$\chi^2_{d.f.}$	34.6_9	38.3_9	103_9	81.2_9	14.6_9	43.8_9	45.6_9	33.7_9	25.5_9	95.8_{27}	44.7_{18}	93.9_{27}	118.6_9	45.6_{18}	73.8_{18}	67.5_9

The Bonferroni correction was applied to the F_{IS} for population calculations.
*P<0.01; **P<0.001.
†F_{IS} and F_{IT} values were significant (P<0.001) for all loci with the exception of LDH-2*.

Table 4 F_{ST} betweenpairedpopulationsof*Sardinapilchardus*.ThenumbersindicatethepopulationsasdescribedinTable1

Population	N	Population								
		1	2	3	4	5	6	7	8	9
1	42	—	—	—	—	—	—	—	—	—
2	35	0.051**	—	—	—	—	—	—	—	—
3	41	0.082***	0.036*	—	—	—	—	—	—	—
4	39	0.088***	0.026NS	0.040*	—	—	—	—	—	—
5	45	0.070***	0.060**	0.053**	0.055**	—	—	—	—	—
6	45	0.096***	0.049**	0.036*	0.032*	0.051**	—	—	—	—
7	69	0.061***	0.044**	0.028*	0.052***	0.041**	0.028*	—	—	—
8	42	0.047**	0.022NS	0.045*	0.041**	0.033*	0.048**	0.039**	—	—
9	45	0.061**	0.026*	0.021NS	0.041**	0.021NS	0.034*	0.022*	0.016NS	—
10	47	0.033*	0.025*	0.048**	0.044**	0.030*	0.052**	0.029**	0.016NS	0.019NS

$*P < 0.05$, $**P < 0.005$, $***P < 0.001$; NS, not significant; N, number of individuals.
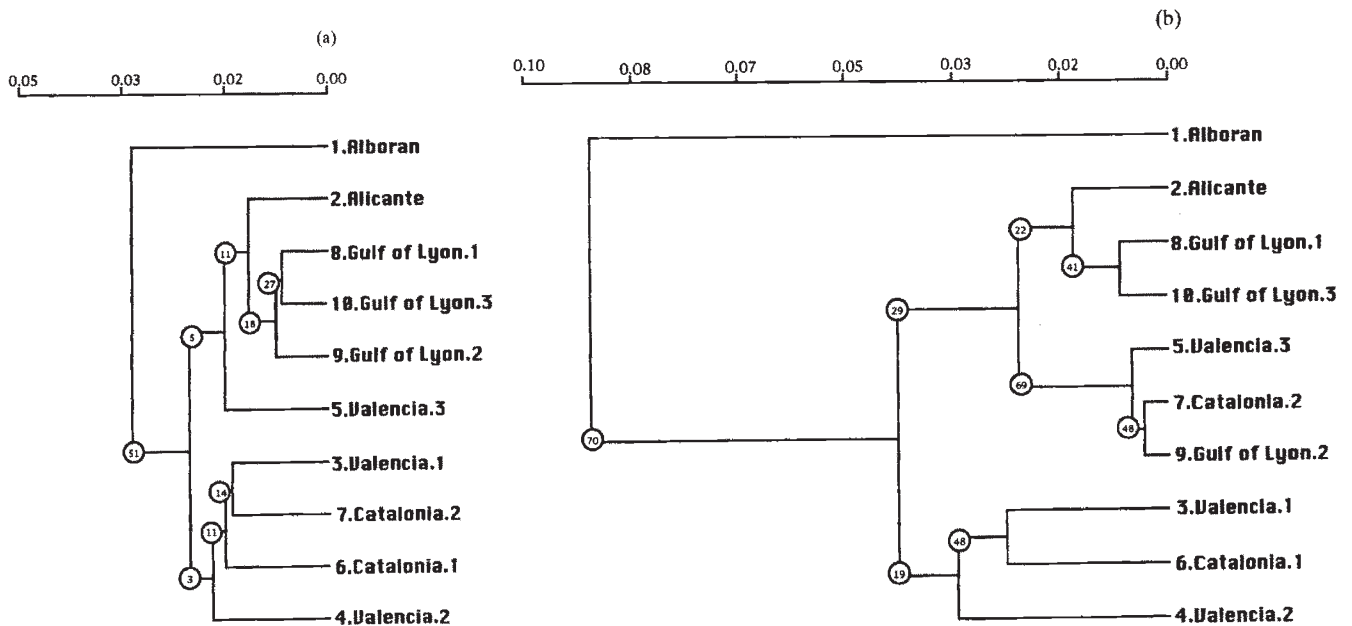
Fig. 2 UPGMA dendrogram for *Sardinia pilchardus* based on the Nei (1972) genetic distance. (a) With all loci. Cophenetic correlation was 0.732. (b) With only the five discriminatory enzymes. Cophenetic correlation was 0.797.

formed by the rest of the loci, with minor variation of frequency (mean SDU = 0.065). A Mann–Whitney U-test between these two was significant (Sokal & Rohlf, 1981). A new cluster was based on Nei's distance with these five variable loci and a bootstrap analysis of this cluster was undertaken (Fig. 2b). This cluster confirmed the Alboran separation and increased the bootstrap percentage value of this separation with respect to the other populations

to 70 per cent, which was a more significant value than that based on all the enzymes.

Discussion

We have used electrophoretic traits to analyse the genetic differences that may exist among the samples of sardine in the western Mediterranean Sea. Although the results obtained for other fish

species in the literature are not directly comparable to the populations of sardines, we have detected more variability than described in the other species. Thus, most enzymatic systems studied in sardine populations are polymorphic, in contrast to studies of other fish species (Ryman, 1983; Wilmot & Burger, 1985; Smith, 1986). This could be a result, in part, of the allozymic loci chosen in this study, but we have found that some loci which are polymorphic in our sardine population study were monomorphic in other species of fishes, for example: ADH^*, IDH^*, PGI^* and CK^* in the Atlantic Salmon (Stähl, 1987), CK^*, MDH^*, PGI^* and SOD^* in sockeye Salmon (Wilmot & Burger, 1985) and LDH^* and PGI^* in anchovy and sardine populations (Spanakis et al., 1989).

A high level of heterogeneity in the Mediterranean population of sardines was shown when the genetic variation was measured as observed heterozygosity, which ranged from 0.110 to 0.160. This range is higher than marine fish data reported by Gyllensten (1985) and Ward et al. (1994) who found similar values of average total heterozygosity (0.063 in six species and 0.064 in 54 species, respectively).

The proportion of genetic diversity attributable to differences among subpopulations can be measured by F_{ST}. More than 60 per cent of marine species show a low population differentiation ($G_{ST} < 0.05$) (Ward et al., 1994), although significant population differences in some of these species have been detected (Smith et al., 1990). In the sardine population we have estimated $F_{ST} = 0.074$, which is similar to the G_{ST} value postulated by Ward et al. (1994) (0.062) and bigger than the 0.042 described by Gyllensten (1985). Following Ward et al. (1994) a $G_{ST} > 0.02$ means that the population is structured. In this way, we can say that the population of the western Mediterranean sardine is structured into genetically differentiated subpopulations.

Alternatively, F_{ST} can be used to derive an estimation of $N_e m$, a reflection of the number of migrants per generation. Using this approach, $N_e m$ was estimated at 3.1. The $N_e m$ estimated by Ward et al. (1994) for freshwater and marine fish corresponds to 0.88 and 3.78, respectively, this latter value being very similar to that found by us in the sardine populations, indicating a similar level of migration.

In the present study the mean heterozygosity observed is lower than expected, as indicated by the global F_{IS}, where the positive values indicate a deficiency in the heterozygotes. This could be a result of inbreeding of genetic cohorts in the samples with the existence of sib relatives, or it could also be a result of the inclusion of more than one genetically differentiated cohort in a single sample.

We have posed two main questions: (i) can the population of sardines in the western Mediterranean be considered as a unique population or, on the contrary, are the samples genetically independent of each other? and (ii) has the Almeria–Oran front affected the populations of sardines?

With respect to the first question, fish species tend to be subdivided into genetically different subpopulations or stocks. Chabanaud (1926) and Lee (1962) recognized two stocks of sardines, one in the Atlantic (Sardina pilchardus pilchardus) and another in the Mediterranean (Sardina pilchardus sardina), as different species, but Svetovidov (1964) considered them only as different races, in accordance with the observations of Furnestin (1952), who also claimed the existence of different races within the Atlantic stock. In the Mediterranean, Laskaridis (1948) recognized four groups in the Greek seas, and Skrivanic & Zavodnik (1973) supported the view that in the Adriatic Sea two major populations of sardines can be found, one in the north and the other in the south, based mainly on hydrographic features of the Adriatic. Spanakis et al. (1989) suggest, in a genetic study concerning Aegean and Ionian populations of sardines, that they do not form an undifferentiated panmictic population, but they exist as semi-independent but not completely isolated breeding units. Our results are in accordance with their hypothesis.

With respect to the second question, the cluster analysis detected a clear displacement of the Alboran population with respect to the rest of the populations; and the F_{ST} between paired populations (Table 4) showed a clear significant difference of Alboran in respect to the other samples. In this area, the mussel Mytilus galloprovincialis exhibits an abrupt discontinuity in allozyme frequencies (Quesada et al., 1995a) which appears to be associated with the Almeria–Oran oceanographic current front (Tintoré et al., 1988), a zone of turbulence in the Alboran Sea, which also may restrict larval dispersal in the oyster Ostrea edulis (Saavedra et al., 1993). Recently, Alemany & Alvarez (1993) studied the same samples of sardines from the ECOMED90 programme, although they compared growth parameters. They observed significant growth differences, greater in the Alboran area than in the other populations and suggested that sardine populations of the Alboran area could belong to the Atlantic subspecies. The authors explained these differences by the action of environmental factors. Of these, mainly upwelling produced by the flux of Atlantic

waters or winds and variation of winter surface temperatures from the Alboran sea to the Gulf of Lyon would be directly related to sardine growth values; although they did not disregard the action of genetic factors, supported by a lack of significant migration. All of these factors are related to the action of Almeria–Oran front.

In order to prove a possible relationship between the genetic variation and geographical distribution we tested the correlation between genetic variation (gene frequencies, heterozygosity) and latitude. Only for the frequency of alleles of LDH-1* did we detect the existence of a cline with an increasing north–south distribution, as can be seen in Fig. 3. The clinal distribution for LDH-1* agrees with the significance of allelic isozyme variation postulated by Powers et al. (1991). These authors detected two alleles for LDH-B in Fundulus heteroclitus with allele b predominant in the northern (colder) cline and a predominant in the southern (warmer) cline. The two isozymes differ in catalytic efficiency, concentration and stability of enzyme. These differences affected the rate of development during the first days of embryonic growth.

With respect to the forces that have established and maintained the differentiation in the population of sardine, apart from the Almeria–Oran front, we think that an initial founder effect, followed by genetic drift within each stock, the existence of genetic flux but lack of significant migration along the coast and environmental adaptations, could be relevant. Although natural selection could act on the LDH-1* polymorphism, functional nonequivalence of allelic products is demonstrated, and it is not

known how selection acts at the molecular level. In relation to the five loci which characterize the separation of the Alboran and the other populations, we do not know how these enzymes could act in this discrimination, and an in-depth study with these enzymes in the future would be necessary to solve this issue.

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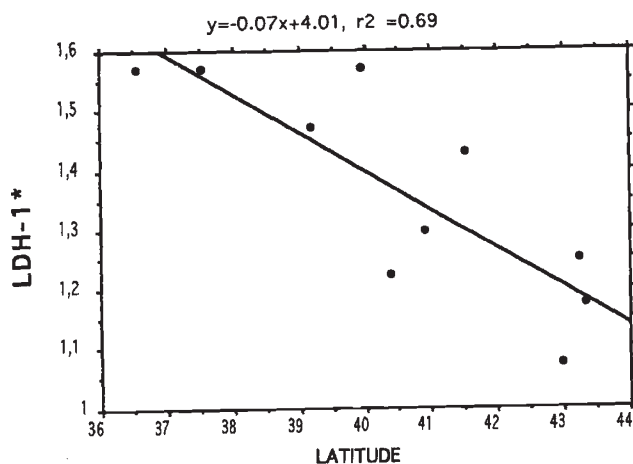

Fig. 3. Regression of the LDH-1* allele frequency and latitude in the different samples of sardines. The allele frequencies have the transformation $\arcsin(\sqrt{p})$. The regression is significant, $P = 0.003$.

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