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# Evidence of a genetic cline for *Sardina pilchardus* along the Northwest African coast

M. Chlaida, V. Laurent, S. Kifani, T. Benazzou, H. Jaziri, and S. Planes

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In all, 700 *Sardina pilchardus* from 14 locations throughout the Atlantic and Mediterranean Moroccan coastlines were screened for eight enzymatic loci that show polymorphism. Analysis of allelic frequencies showed a genetic break in the area of the Bay of Agadir, segregating two groups of samples, confirmed by analysis of molecular variance; a first consisting of Sidi Ifni (29°12'N) and southern samples down to the southern limit of the species in Mauritania (19°03'N 16°28'W), and a second starting in the Bay of Agadir (30°48'N) and consisting of northern samples that appeared homogeneous with the Mediterranean samples. Specifically, a survey of the *SOD\** loci demonstrated a genetic cline in allelic frequencies along the coastline. Using a clinal variation model under a state of equilibrium, we estimate a dispersal rate of  $189 \pm 52$  km per generation between Mauritania and Ras Kebdana. This major break towards the south of the Bay of Agadir offers a new perspective on the management of sardine, which ideally ought now to be managed as two stocks along the Moroccan coast. The break is most likely induced by hydrodynamic influences that virtually isolate southern from northern populations.

**Keywords:** allozymes, genetic cline, marine fish, Northwest Africa, *Sardina pilchardus*.

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## Introduction

A major objective of fisheries genetics is to identify discrete populations or groups with more or less restricted gene flow (Carvalho and Hauser, 1995; Hauser and Ward, 1998). Unlike terrestrial environments, the marine realm is generally regarded as lacking major geographical barriers to dispersal and gene flow, leading to large, homogeneous populations. In addition, many marine organisms have a large population size, high fecundity, and excellent dispersal capabilities as planktonic larvae and free-swimming adults. Limited genetic differentiation between geographic populations would therefore be expected, because of the high levels of gene flow facilitated by such features (Norris, 2000). In populations of pelagic fish, even less genetic structure is expected, because adults often disperse over large distances. Populations of pelagic fish are often therefore unfragmented over large areas, because groups of fish can migrate thousands of kilometres (Begg *et al.*, 1998; Chikhi *et al.*, 1998; Laurent *et al.*, 2007), usually leading to weak genetic differentiation (Ward *et al.*, 1994; D'Amato and Carvalho, 2005). For some populations of pelagic fish, genetic differentiation can result from breaks in the hydrography, such as the Almeria–Oran front that limits gene flow (Patarnello *et al.*, 2007), or from environmental conditions that can change the effective size of a population and/or its reproductive success (Gaggiotti and Vetter, 1999). In this context, a genetic break in marine pelagic species provides an opportunity to understand

mechanisms influencing population structure, along with the consequences for fisheries management.

The European sardine (*Sardina pilchardus*) is found from the Celtic Sea and North Sea south to Mauritania, with populations in the Mediterranean Sea and the Black Sea. Populations of Madeira, the Azores and the Canary Islands are at the western limit of the distribution (Parrish *et al.*, 1989). Occasionally, populations may be found as far south as Senegal during episodes of low water temperature (Corten and van Kamp, 1996; Binet *et al.*, 1998). Recent genetic surveys failed to detect any genetic differences among sardine samples in the Bay of Biscay, although a cline was observed for two loci (*PGM-1\**, *PEP-LT\**), using multi-locus heterozygosity and geographical data, i.e. longitude (Laurent *et al.*, 2006). Additional genetic results emphasize a low effective population size despite a large total population (Laurent and Planes, 2007). However, Chlaida *et al.* (2005) demonstrated a break in the genetic structure in the area of the Bay of Agadir (Morocco), a break mainly resulting from a discontinuity in the allelic frequencies of the *SOD\** allozyme.

Our most recent analysis was conducted on samples from seven stations ( $n = 251$ ). Two enzymes were surveyed (*LDH\** and *SOD\**), but only *SOD\** was polymorphic and highly informative. Statistical analysis indicated significant heterogeneity between samples. A homogeneous population was identified in the south, from Tarfaya to Dakhla (24–28°N). The Agadir (30°N) population appeared to overlap with others. The population identified

offshore of Larache (35°N) was similar to the population located off southern Portugal (37°N; Chlaida *et al.*, 2005). We note too that there is a genetic break among mussel populations in this area (Jaziri and Benazzou, 2002). Considering the interest in the area and the value of the fishery for sardine, we focused our work along the Moroccan coast to describe the genetic shift better, and to investigate the evolutionary and biological processes responsible for the population structure.

## Material and methods

### Sampling

In all, 14 samples of 50 sardine were collected (Figure 1). Of these samples, ten were collected along the coast using the RV “Dr Fridtjof Nansen” in December 2004, three in the harbours of Larache, M’dik, and Ras Kebdana, and a last one was collected in April 2005 (Cadiz) using a Portuguese research cruise.

Fish were dissected to isolate the liver and a piece of muscle. Each piece of tissue was ground manually on crushed ice in 1 ml of distilled water, to limit the degradation of enzymatic activity. Homogenates were subdivided into four Eppendorf tubes and stored at  $-30^{\circ}\text{C}$ .

### Allozyme electrophoresis

Samples were processed by horizontal starch gel electrophoresis, following the technique of Pasteur *et al.* (1987), using three buffers (TC 8.0, TC 8.5 and TG 9.0). In all, eight enzymatic systems were screened: *SOD*\* [EC.1.15.1.1] on TG 9.0; *PGM*\* [EC 2.7.5.1], *IDH*\* [1.1.1.42], *AAT*\* [EC 2.6.1.1], *MDH*\* [1.1.1.37], *ME*\* [EC 1.1.1.40], *MPI*\* [5.3.1.8] on TC 8.0, and *EST*\* [EC 2.6.11] on TC 8.5. The reader is referred to Shaklee *et al.* (1990) for locus nomenclature. In total, we sampled ten loci, including loci for *ME*\* and *MDH*\*. Alleles and genotypes were scored according to Shaklee *et al.* (1990), in which the most common allele in the first sample was designated as 100. The other variants were subsequently numbered according to their electrophoretic mobilities relative to the most common allele.

### Statistical analysis

Allelic and genotypic frequencies of polymorphic loci were obtained by counting phenotypes directly from the gels and analysing them with GENETIX 4.05 (Belkhir *et al.*, 1996–2004; <http://www.univ-montp2.fr/genome-pop/genetix.htm>). Each sample’s genotypic frequency deviation from Hardy–Weinberg equilibrium was assessed with the fixation index ( $F_{IS}$ ).

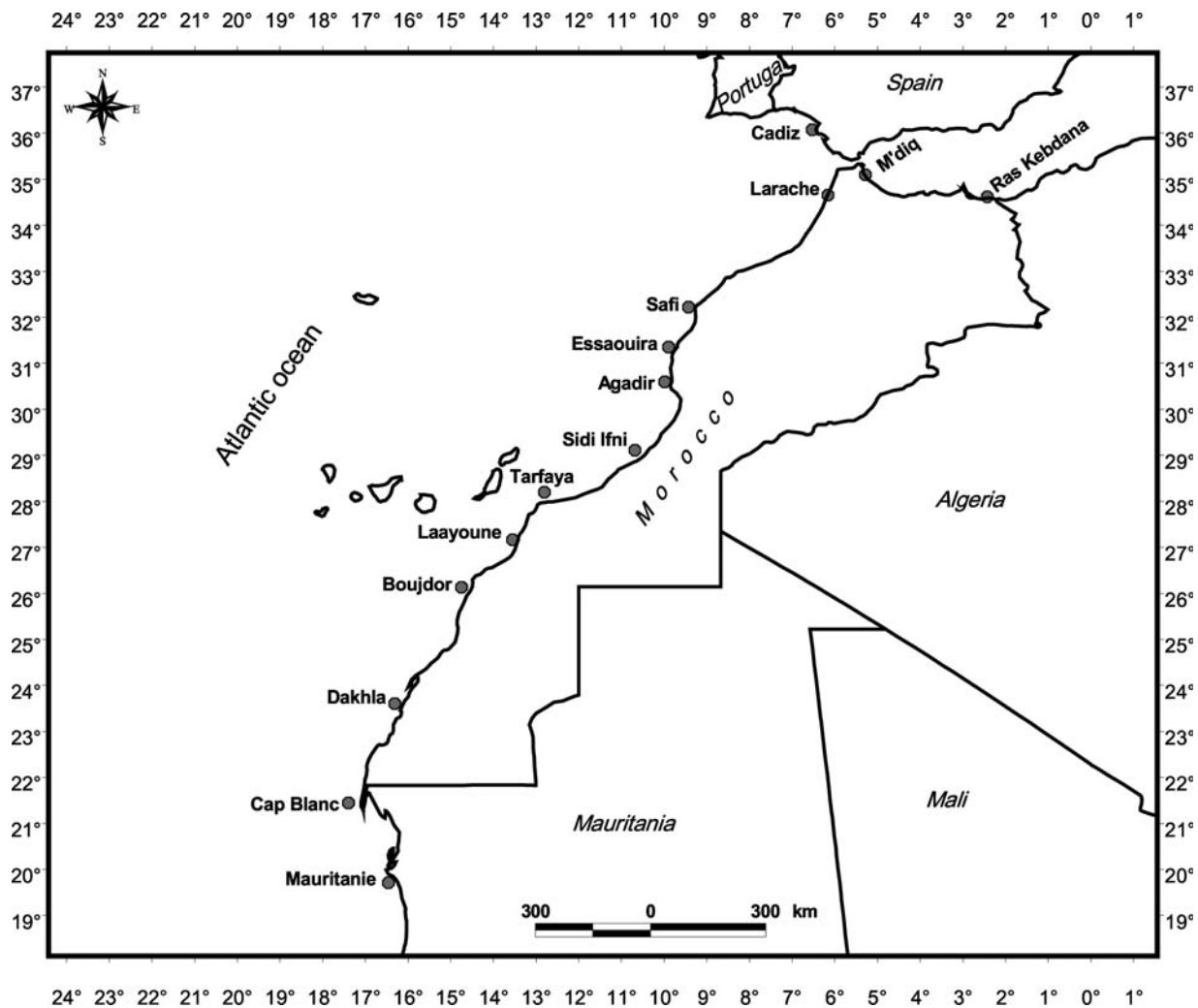


Figure 1. Map of the sampling locations off Northwest Africa.

Multilocus deviation from Hardy–Weinberg equilibrium was tested statistically using the Markov chain method implemented in GENEPOP 3.4 (Raymond and Rousset, 1995). Significance levels for statistical tests were adjusted for each sample with a sequential Bonferroni procedure (Rice, 1989). Genetic divergence between samples or populations was assessed with Wright's standardized variance in allelic frequencies ( $F_{ST}$ ) (Wright, 1969). This index was computed over all samples, following the Weir and Cockerham (1984) algorithm available on GENETIX.

Genetic structure was explored by computing a neighbour-joining tree with groups tested using the hierarchical analysis of molecular variance (AMOVA) implemented in ARLEQUIN 2.000 (Excoffier *et al.*, 1992; Schneider *et al.*, 2000). This provided estimates of the percentage of total variance accounted for within and among groups of samples. The basic objective was to find a maximum variance among groups, and a minimum within, to test whether groups aggregated in genetically similar samples of isolated major genetic units. Statistical significance was determined by more than 1000 permutations of genotypes.

The correlation between geographic distance separating sites and genetic differentiation (multilocus  $F_{ST}$ ) was performed over all loci using Mantel's test, based directly on the distribution of 10 000 randomized matrices computed by a permutation (Mantel, 1967). Mantel's tests were computed using the MANTEL procedure in GENETIX. We followed the Hutchinson and Templeton (1999) model that directly links  $F_{ST}$  with geographic distance for these correlations.

We computed  $\sigma$ , the dispersal rate per generation needed to produce a linkage disequilibrium in the centre of a cline, from  $\sigma = w\sqrt{Dr}$ , according to Szymura and Barton (1986). In this estimate,  $w$  is the cline width,  $D$  the average linkage disequilibrium in the centre of the cline, and  $r = 0.5$  for unlinked loci. Linkage disequilibrium values ( $D$ ) are constrained by allele frequencies, and they decrease in populations that show a small number of polymorphisms. Therefore, we used the correlation coefficient  $R$  between loci,  $R = D/\sqrt{pquv}$ , as the linkage disequilibrium value, which lies between  $-1$  and  $+1$  (Szymura and Barton, 1986), and obtained the equation  $\sigma = w\sqrt{(Rr)/4}$ . Two methods estimate  $w$ , the width of the clines. Following Szymura and Barton (1986), the width of the cline was the geographic distance including 20 and 80% of parental genotype. This method implied that we sampled the entire cline. The second method, that of Barton and Gale (1993), proposes that width is the inverse slope of the cline if allelic frequencies are shifted from 0 to 1. If not, then the estimate of width is corrected by  $w = \Delta p/\text{slope}$ . Linkage disequilibria and correlation coefficients were estimated using the LINKDIS program (Black and Krasfur, 1985) implemented in GENETIX. It was assumed that diploid genotypes were in Hardy–Weinberg proportion, to reduce the problem to an estimation of haploid gamete frequencies. Moreover, the third and higher order disequilibria were considered to be zero, reducing the computations to pairwise comparisons among all interpretable loci. Linkage disequilibrium was statistically tested using the Markov chain method implemented in GENEPOP 3.4.

## Results

Among the loci, only  $SOD^*$  and  $PGM^*$  were highly polymorphic, whereas  $IDH^*$ ,  $AAT^*$ , and  $EST^*$  showed low levels of polymorphism, and  $ME-1^*$ ,  $ME-2^*$ ,  $MDH-1^*$ ,  $MDH2^*$ , and  $MPI^*$  were monomorphic (Table 1). All samples were in Hardy–Weinberg equilibrium ( $F_{IS} = 0.018$ ,  $p = 0.128$ ). The overall genetic

differentiation was highly significant, with  $F_{ST} = 0.205$  ( $p < 0.000$ ). From the pairwise genetic differences matrix (Table 2) and the neighbour-joining tree (Figure 2), we noted a segregation of two groups: (i) Sidi Ifni, Tarfaya, Laayoune, Dakhla, Cap Blanc, and Mauritania, and (ii) Cadiz, Larache, Safi, Essaouira, and Agadir from the Mediterranean, expressing only limited differences between Atlantic and Mediterranean samples. The AMOVA of these two groups explained 29.58% of the total variance and was highly significant ( $p < 0.001$ ; Table 3). In contrast, this AMOVA showed that, while being significant ( $p < 0.01$ ), just 2.15% of the total variance was contained within groups, suggesting some minor differentiation within the two groups.

Separate analysis of each locus indicated that most of the variation was attributable to allelic frequency differences in  $SOD^*$ . In  $SOD^*$ , we demonstrated a significant relationship between allele  $Sod^*-100$  and latitude ( $r^2 = 0.78$ ,  $p < 0.001$ ) and longitude ( $r^2 = 0.878$ ,  $p < 0.001$ ; Figure 3).  $Sod^*-100$  frequencies were similar from Mauritania to Tarfaya, and from Essaouira to Cadiz, with a major transition between Sidi Ifni and Agadir. Frequencies in Mediterranean samples also appear to be different from Atlantic frequencies, suggesting another break near the Strait of Gibraltar. Finally, Mantel's test demonstrated a significant relationship between geographic distance and pairwise genetic differentiation among all samples ( $r = 0.80$ ,  $p = 0.0003$ ).

Based on clines of two alleles,  $Sod^*-100$  and  $Sod^*-80$  frequencies, we estimated the width of the two clines (i.e. the length of the two transition zones). According to the Barton and Gale (1993) model, we described a theoretical width of the slope of 2066 km between Mauritania ( $19^\circ 03'N$   $16^\circ 28'W$ ) and Ras Kebdana ( $35^\circ 09'N$   $02^\circ 26'W$ ). All samples were pooled within a single population, and the correlation coefficient ( $r$ ) between  $SOD^*$  and  $PGM-1^*$  for the cline was 0.067 ( $p = 0.05$ ). Based on  $w$  and the computed  $R$ -value, we estimated the dispersal rate to be  $\sigma = 189 \pm 52$  km per generation, representing  $\sim 9.1\%$  of the cline width.

To verify whether the  $SOD^*$  locus is under selection, we followed Beaumont and Nichols (1996; Figure 4), a method based on the distribution of  $F_{ST}$  conditional on gene diversity rather than allele frequency. They generated, by simulation, the 95% confidence intervals for  $F_{ST}$  under distinct genetic models, then the estimates of  $F_{ST}$  at different loci were plotted against their expected gene diversities. Outlier loci were considered under selective regime (Guinand *et al.*, 2004). The test shows that no polymorphic locus deviates from the expected under neutrality.

## Discussion and conclusions

This analysis of the genetic structure of *S. pilchardus* from the Atlantic and Mediterranean Moroccan coast based on allozyme markers demonstrated strong heterogeneity among samples ( $F_{ST} = 0.205$ ). Such strong differentiation among populations is unusual in pelagic species (Bembo *et al.*, 1996; Ramon and Castro, 1997; Cimmaruta *et al.*, 2005), and it raises questions of its origin and maintenance through time. In this study, the genetic differentiation is attributable to a single locus,  $SOD^*$ , that has been used before in *S. pilchardus* to discriminate fish stocks (Ramon and Castro, 1997; Chlaida *et al.*, 2005; Laurent *et al.*, 2007), and all other loci screened were almost monomorphic. In fact,  $SOD^*$  alone discriminates the break in the Agadir area, as well as the break at the Strait of Gibraltar. In addition, all gradients, e.g. longitudinal, latitudinal, and distance, are exclusively driven by  $SOD^*$  allelic frequencies.

**Table 1.** Allelic frequencies, observed heterozygosity, and  $F_{IS}$  values for the samples of *S. pilchardus*.

Locus	Allele	Sample													
		Mauritanie	Cap Blanc	Dakhla	Bojdor	Laayoune	Tarfaya	Sidi Ifni	Agadir	Essaouira	Safi	Larache	Cadiz	M'dik	Ras Kebdana
<i>SOD*</i>															
<i>n</i>		50	50	30	50	50	50	50	43	49	50	49	49	42	46
	80	0.06	0.04	0.07	0.04	0.09	0.08	0.23	0.33	0.48	0.44	0.46	0.46	0.62	0.69
	100	0.94	0.96	0.93	0.96	0.91	0.92	0.77	0.66	0.52	0.56	0.54	0.54	0.38	0.31
<i>EST*</i>															
<i>n</i>		50	50	30	50	50	50	50	50	46	50	50	37	43	49
	100	1.00	1.00	1.00	1.00	0.99	1.00	0.99	1.00	0.97	0.97	1.00	1.00	0.98	0.99
	110	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.03	0.03	0.00	0.00	0.02	0.01
<i>IDH*</i>															
<i>n</i>		50	50	30	50	50	50	50	50	47	50	50	50	43	46
	60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.097	0.99	1.00	1.00	1.00	1.00
<i>PGM*</i>															
<i>n</i>		50	50	30	50	50	50	50	50	50	50	50	50	43	49
	100	0.97	0.94	0.97	0.95	0.97	0.96	0.95	0.99	0.97	0.97	0.99	0.99	0.99	0.99
	150	0.03	0.06	0.03	0.05	0.03	0.04	0.05	0.01	0.03	0.03	0.01	0.01	0.01	0.01
<i>AAT*</i>															
<i>n</i>		50	50	30	50	50	50	50	50	50	50	50	50	43	50
	85	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	100	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>ME*</i>															
<i>n</i>		50	50	30	50	50	50	50	50	50	50	50	50	43	50
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>MDH*</i>															
<i>n</i>		50	50	30	50	50	50	50	50	50	50	50	50	43	50
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>MPI*</i>															
<i>n</i>		50	50	30	50	50	50	50	50	50	50	50	50	43	50
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
$H_{obs}$		0.022	0.022	0.025	0.022	0.032	0.03	0.062	0.063	0.092	0.087	0.061	0.056	0.050	0.051
$F_{IS}$		-0.042	0.150	-0.041	-0.037	-0.065	-0.061	-0.056	-0.077	-0.071	-0.102	0.062	0.104	0.262	0.141
	1	0.428	1	1	1	1	1	1	-	0.296	0.93	-	-	0.017	-

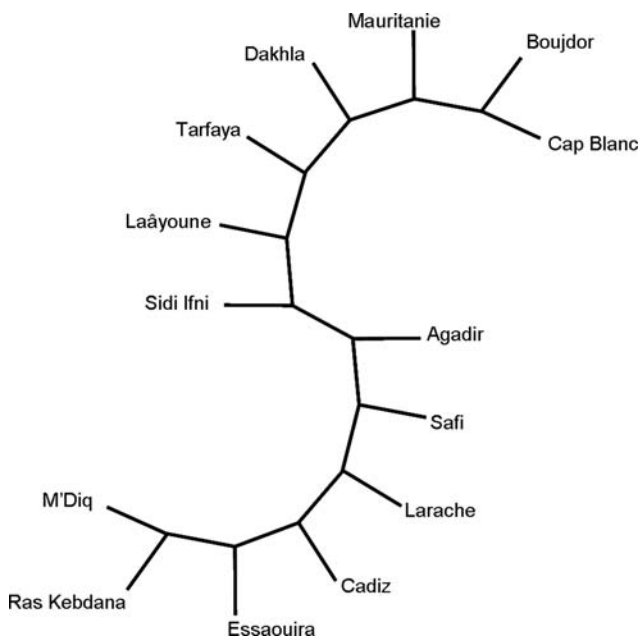
**Table 2.**  $F_{ST}$  pairwise values for all sites, with emboldened values representing statistically significant differences ( $p < 0.001$ ).

Site	Cap Blanc	Dakhla	Bojdor	Laayoune	Tarfaya	Sidi Ifni	Agadir	Essaouira	Safi	Larache	Cadiz	M'dik	Ras Kebdana
Mauritanie	-0.003	-0.013	-0.005	-0.005	-0.007	0.075	<b>0.193</b>	<b>0.290</b>	<b>0.260</b>	<b>0.311</b>	<b>0.311</b>	<b>0.473</b>	<b>0.549</b>
Cap Blanc		-0.007	-0.010	0.005	-0.001	0.088	<b>0.211</b>	<b>0.301</b>	<b>0.272</b>	<b>0.323</b>	<b>0.323</b>	<b>0.477</b>	<b>0.551</b>
Dakhla			-0.007	-0.010	-0.012	0.057	<b>0.163</b>	<b>0.250</b>	<b>0.223</b>	<b>0.273</b>	<b>0.272</b>	<b>0.428</b>	<b>0.508</b>
Bojdor				0.005	-0.001	0.093	<b>0.220</b>	<b>0.310</b>	<b>0.281</b>	<b>0.334</b>	<b>0.334</b>	<b>0.492</b>	<b>0.565</b>
Laayoune					-0.008	0.044	<b>0.144</b>	<b>0.243</b>	<b>0.213</b>	<b>0.258</b>	<b>0.257</b>	<b>0.419</b>	<b>0.496</b>
Tarfaya						0.053	<b>0.159</b>	<b>0.258</b>	<b>0.228</b>	<b>0.274</b>	<b>0.274</b>	<b>0.435</b>	<b>0.512</b>
Sidi Ifni							<b>0.018</b>	<b>0.092</b>	0.067	<b>0.090</b>	0.089	<b>0.225</b>	<b>0.300</b>
Agadir								0.028	0.012	0.019	0.018	0.126	<b>0.196</b>
Essaouira									-0.006	-0.005	-0.006	0.021	0.061
Safi										-0.007	-0.007	0.041	0.090
Larache											-0.011	0.035	0.083
Cadiz												0.034	0.082
M'dik													-0.005

A genetic cline, i.e. a geographic zone with differentiated interbreeding populations, has been described in few cases in the marine ecosystem (e.g. Duggins *et al.*, 1995; Hare and Avise, 1996; Planes and Doherty, 1997; Sotka *et al.*, 2004). In fact, clines may represent an evolutionary balance between selection and dispersal, so long as the stability of the cline in time and space can be assumed (Barton and Hewitt, 1985; Barton and Gale, 1993). For the European sardine, strong genetic divergence was described by Chlaida *et al.* (2005), and it is likely that the actual steep break-down in allelic frequencies of  $SOD^*$  in the area of Agadir is stable at least over short time-scales. Currently, the significant relationship between geographic distance and pairwise genetic differentiation statistically validated by Mantel's test ( $r = 0.80$ ,  $p = 0.0003$ ) reflects a spatial gradient in the genetic

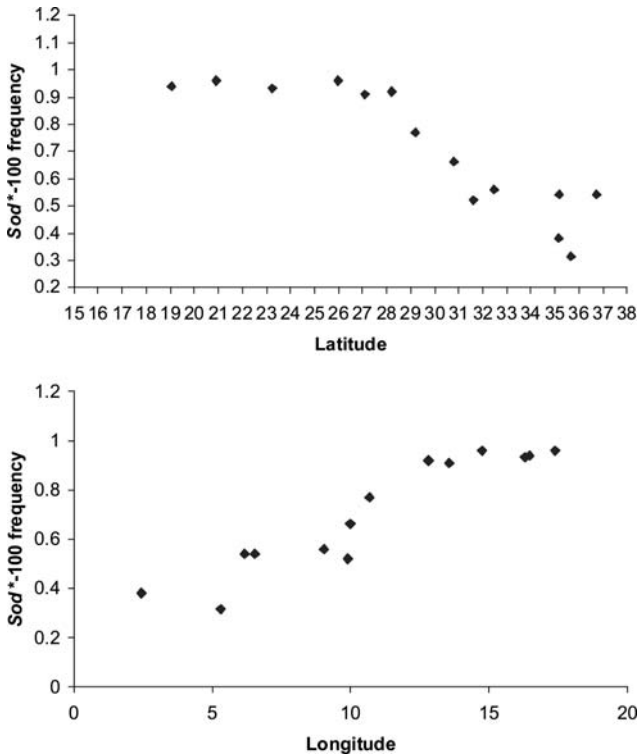
differentiation compatible with cline shape and isolation by distance models, which we discuss below.

Overall, given the high fecundity, large population size, and potential migration capability across the lifespan of the sardine, the strong divergence between southern and northern areas of the Moroccan coast can only result from a scenario involving isolation of a southern peripheral population at some stage in ancestral time, with subsequent reunion in the main northern distribution area. Even considering such a historical scenario, maintenance of the current situation requires significant limitation in the gene flow. Part of the explanation is certainly related to the hydrodynamics in the area, with complicated upwelling and gyres along the southern Moroccan coast, in addition to the previously identified barriers of the Strait of Gibraltar and the Alboran Sea in the north, which together serve to limit gene flow. Along the Atlantic coast of Morocco, the vertical distributions in the northern area (Cap Sim to Cap Ghir) ( $31^{\circ}30' - 30^{\circ}37'N$ ) and the southern area between Sidi Ifni ( $29^{\circ}12'N$ ) and Cap Draa ( $\sim 29^{\circ}N$ ) are different (Johnson and Stevens, 2000; Stevens and Johnson, 2003). In the northern part, the upwelling is situated between Cap Sim and Cap Tafelney ( $32^{\circ}30' - 31^{\circ}06'N$ ). The bottom topography allows a southwest extension from this tongue of cold water, rich in nutrients. Between this

**Figure 2.** Neighbour-joining dendrogram of genetic distance for the  $SOD^*$  locus using the distance matrix of Nei (1978).**Table 3.** AMOVA analysis comparing two groups of *Sardina pilchardus*.

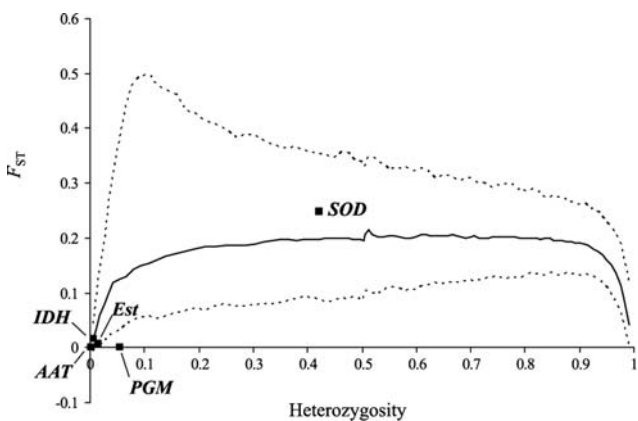
Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	$p$ -values
Among groups	1	56.24	0.08	29.58	0
Among populations within groups	12	9.17	0.01	2.15	0
Within populations	1 332	253.45	0.19	68.27	0.00
Total	1 345	318.86	0.28		

Group 1 included samples from Cadiz, Larache, Safi, Essaouira, Agadir, Ras Kebdana, and M'dik, and Group 2 included samples from Sidi Ifni, Tarfaya, Laayoune, Boujdor, Dakhla, Cap Blanc, and Mauritania.



**Figure 3.** The relationship between *Sod\**-100 frequency and latitude (top panel) and between *Sod\**-100 frequency and longitude (bottom panel).

tongue and the coast, a countercurrent transports warmer water from the Bay of Agadir to the north of Cap Ghir (Minas *et al.*, 1982). In the southern area, between Sidi Ifni and Cap Juby (~28°N), the continental shelf is wider and has a gentler slope. The winds are more regular but intermediate in strength, with means falling between the maxima and the minima of the northern area.



**Figure 4.** Distribution of per-locus  $F_{ST}$  values as a function of heterozygosity. Dotted lines denote 0.95 and 0.05 quantiles estimated from simulation conducted in the *Fdist* program, and the solid line represents the median. Each dot represents a locus. Dots above the upper dotted line may be classified as outliers potentially under divergent selection, and dots below the bottom dotted line as outliers potentially under balancing selection. Here, only one locus is found outside the confidence interval, *PGM\**, which appears below the bottom dotted line.

The cold waters of the upwelling appear at the surface only occasionally. From Cap Ghir to Cap Juby (roughly 30°37'N to 28°N), the coastline has a prominent concavity. This, together with the morphology of the Atlas range in this region, causes the upwelling to be reduced, even during summer. The Cap Ghir filament (~30°N), which stretches offshore many hundreds of kilometres into the open ocean, persists year-round. Pelegrí *et al.* (2005) suggest that, in autumn, the Cap Ghir filament represents a major separation of the Canary Current from the coast, potentially exporting large quantities of organic material into the open ocean. Near Cap Juby, the Canaries archipelago introduces large mesoscale variability, mainly in the form of downstream vortices (Aristegui *et al.*, 1994) that interact with the Cap Juby filament system and enhance cross-slope exchanges. Such structure would lead to a strong, stable hydrodynamic barrier in the area of Agadir that would explain the major break in the genetic structure of the European sardine. At the Strait of Gibraltar, the barrier to gene flow appears more flexible, because there is not such a reduction in the allelic frequencies of *SOD\**. In fact, the break in the Alboran Sea has been described previously, with the existence of transition zones for several species, e.g. sea bass (*Dicentrarchus labrax*; Naciri *et al.*, 1999), mussels (*Mytilus galloprovincialis*; Quesada *et al.*, 1995), and other marine species (Borsa *et al.*, 1999, Bonhomme *et al.*, 2002). The break was initially the result of the isolation of the Mediterranean population when sea levels were much lower, but it is now maintained by the current bathymetric frame that limits water exchange through the narrow strait. This evidence suggests that hydrodynamic influences probably explain the genetic structure of sardine along the Moroccan coast of the Atlantic, and at the Strait of Gibraltar and in the Mediterranean.

However, based on the equilibrium model of cline, it is difficult to understand the steep change in *SOD\** frequencies in the area of Agadir, assuming that the barrier to gene flow is only oceanographic. The basic theory of clines superimposes the idea of secondary contact between initially isolated populations, but in the context of an evolutionary balance between selection and dispersal. We showed that, for Agadir, the dispersal is likely to be limited, so the selection process is also likely to be only marginal to counter-balance the limited dispersal (Sotka and Palumbi, 2006). From our data, it is not possible to evaluate the selection because we have only one locus showing significant heterozygosity. Therefore, we could not test any linkage disequilibrium that could be viewed as potentially resulting from selective processes. In fact, as mentioned previously, it is only *SOD\** that drives the cline. *SOD\** has been shown to act in differentiation of populations of pelagic species such as tunas (*Thunnus* spp.) in the Mediterranean (Pujolar *et al.*, 2003). However, it is difficult to generate an outcome regarding the effect of various alleles of *SOD\** because this locus may not be the one under selection and it could result from a hitch-hiking process. Potential selective processes have already been emphasized in clupeoids (Magoulas *et al.*, 1996; Tudela *et al.*, 1999) and other marine species (Mallet and Barton, 1989; Borsa *et al.*, 1999; Riginos *et al.*, 2002; Grant *et al.*, 2006).

Assuming no selective process and considering the entire width of the cline, we estimate a dispersal rate of 189 km per generation. This value has, in fact, little meaning for, on the one hand, it is impossible to estimate the effect of selection, and on the other, we consider the width of the cline as being the entire Atlantic coast of Morocco (~2000 km), whereas most of the transition

in allelic frequencies is between Sidi Ifni and Agadir, which are separated by ~200 km. With a tenfold reduction in the dispersal rate for a pelagic species such as sardine, this would probably mean that the selective process is much stronger. However, the test for selection (Beaumont and Nichols, 1996; Figure 4) did not reveal any particular trends in *SOD\**, refuting the idea that the locus was under selection. However, the result of this test must be considered with caution because it was computed with just three loci, decrease its power. Further work regarding the real effect of *SOD\** should be undertaken to identify patterns through year classes, e.g. comparing young-of-the-year with adults, and larvae with adults, and investigating any changes through time.

The present work has clearly demonstrated two breaks in genetic population structure that should be considered by fishery managers as yielding independent stocks. Genetic surveys of pelagic species have rarely been able to identify different stocks. This is clearly an important distinction that should be kept in consideration during subsequent management decisions for pelagic species off Northwest Africa. In terms of maintenance of these stocks, the processes underlying the patterns seem to be more related to hydrographic features in the area between Agadir and Sidi Ifni. Moreover, this study has shown that the apparent oceanic barrier is functional for sedentary species, as evidenced for mussels by Jaziri and Benazzou (2002), and for migratory species such as sardine. However, owing to the limited number of polymorphic loci, more work is needed, and an analysis of change over time in each region is needed to confirm the results.

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### References

- Aristegui, J., Sangrà, P., Hernández-León, S., Canton, M., Hernández-Guerra, A., and Kerling, L. 1994. Islands-induced eddies in the Canary Islands. *Deep Sea Research*, 41: 1509–1525.
- Barton, N. H., and Gale, K. S. 1993. Genetic analysis of hybrid zones. *In Hybrid Zones and the Evolutionary Process*, pp. 13–45. Ed. by R. Harrison. Oxford University Press, New York.
- Barton, N. H., and Hewitt, G. M. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, 16: 113–148.
- Beaumont, M. A., and Nichols, R. A. 1996. Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London, Series B*, 263: 1619–1626.
- Begg, G. A., Keenan, C. P., and Sellin, M. J. 1998. Genetic variation and stock structure of school mackerel and spotted mackerel in northern Australian waters. *Journal of Fish Biology*, 53: 543–559.
- Belkhir, K., Borsa, P., Chiki, L., Goudet, J., and Bonhomme, F. 1996–2004. Genetix version 4, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier, France.
- Bembo, D. G., Carvalho, G. R., Cingolani, N., and Pitcher, T. J. 1996. Electrophoretic analysis of stock structure in northern Mediterranean anchovies, *Engraulis encrasicolus*. *ICES Journal of Marine Science*, 53: 115–128.
- Binet, D., Samb, B., Taleb Sidi, M., Levenez, J. J., and Servain, J. 1998. Sardines and other pelagic fisheries changes associated with trade wind increases in the Canary Current upwelling (26°N–14°N), late 1960s–early 1990. *In Global versus Local Changes in Upwelling Systems*, pp. 211–233. Ed. by M. H. Durand, R. Mendelsohn, P. Cury, C. Roy, and D. Pauly. Orstom, Paris.
- Black, W. C., and Krasfur, E. S. 1985. LINKDIS Software. Department of Entomology, University of Iowa, Iowa City.
- Bonhomme, F., Naciri, M., Bahri, L., and Lemaire, C. 2002. Analyse comparée de la structure génétique de deux espèces de poissons marins apparentées et sympatriques *Dicentrarchus labrax* et *Dicentrarchus punctatus*. *Comptes Rendus de l'Académie des Sciences, Biologies*, 325: 213–220.
- Borsa, P., Daguin, C., Caetano, S. R., and Bonhomme, F. 1999. Nuclear-DNA evidence that northeastern Atlantic *Mytilus trossulus* mussels carry *M. edulis* genes. *Journal of Molluscan Studies*, 65: 504–507.
- Carvalho, G. R., and Hauser, L. 1995. Molecular genetics and the stock concept in fisheries. *In Molecular Genetics in Fisheries*, pp. 55–80. Ed. by G. R. Carvalho, and T. J. Pitcher. Chapman and Hall, London.
- Chikhi, L., Bonhomme, F., and Agnese, J. F. 1998. Low genetic variability in a widely distributed and abundant clupeid species, *Sardinella aurita*. New empirical results and interpretations. *Journal of Fish Biology*, 52: 861–878.
- Chlaida, M., Kifani, S., Lenfant, P., and Ouragh, L. 2005. First approach for the identification of sardine populations *Sardina pilchardus* (Walbaum 1792) in the Moroccan Atlantic by allozymes. *Marine Biology*, 149: 169–175.
- Cimmaruta, R., Bondanelli, P., and Nascetti, G. 2005. Genetic structure and environmental heterogeneity in the European hake *Merluccius merluccius*. *Molecular Ecology*, 14: 2577–2591.
- Corten, A., and van Kamp, G. 1996. Variation in the abundance of southern fish species in the southern North Sea in relation to hydrography and wind. *ICES Journal of Marine Science*, 53: 1113–1119.
- D'Amato, M. E., and Carvalho, G. R. 2005. Population genetic structure and history of the long-tailed hake *Macruronus magellanicus*, in the SW Atlantic as revealed by mtDNA RFLP analysis. *ICES Journal of Marine Science*, 62: 247–255.
- Duggins, C. F., Karlin, A. A., Mousseau, T. A., and Relyea, G. 1995. Analysis of a hybrid zone in *Fundulus majalis* in a northeastern Florida ecotone. *Heredity*, 74: 117–128.
- Excoffier, L., Smouse, P., and Quattro, J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131: 479–491.
- Gaggiotti, O. E., and Vetter, R. D. 1999. Effect of life history strategy, environmental variability, and overexploitation on the genetic diversity of pelagic fish populations. *Canadian Journal of Fisheries and Aquatic Sciences*, 56: 1376–1388.
- Grant, W. S., Spies, I. B., and Canino, M. F. 2006. Biogeographic evidence for selection on mitochondrial DNA in North Pacific walleye pollock *Theragra chalcogramma*. *Journal of Heredity*, 97: 571–580.
- Guinand, B., Lemaire, C., and Bonhomme, F. 2004. How to detect polymorphisms undergoing selection in marine fishes? A review of methods and case studies, including flatfishes. *Journal of Sea Research*, 51: 167–182.
- Hare, M. P., and Avise, J. C. 1996. Molecular genetic analysis of a stepped multilocus cline in the American oyster (*Crassostrea virginica*). *Evolution*, 50: 2305–2315.
- Hauser, L., and Ward, R. D. 1998. Population identification in pelagic fish: the limits of molecular markers. *In Advances in Molecular Ecology*, pp. 191–224. Ed. by G. Carvalho. IOS Press, Amsterdam.
- Hutchinson, D. W., and Templeton, A. R. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the



- relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, 53: 1898–1914.
- Jaziri, H., and Benazzou, T. 2002. Différenciation allozymique multi locus des populations de moule *Mytilus galloprovincialis* des côtes marocaines. *Comptes Rendus Biologie*, 325: 1175–1183.
- Johnson, J., and Stevens, I. 2000. A fine resolution model of the eastern North Atlantic between the Azores, the Canary Islands and the Gibraltar Strait. *Deep Sea Research I*, 47: 875–899.
- Laurent, V., Magoulas, A., Caneco, B., and Planes, S. 2007. Isolation by distance and selection effects on genetic structure of sardines *Sardina pilchardus* Walbaum. *Journal of Fish Biology*, 71: 1–17.
- Laurent, V., and Planes, S. 2007. Effective population size estimation on *Sardina pilchardus* in the Bay of Biscay using a temporal genetic approach. *Biological Journal of the Linnean Society*, 90: 591–602.
- Laurent, V., Voisin, M., and Planes, S. 2006. Genetic clines in the Bay of Biscay provide estimates of migration for *Sardina pilchardus*. *Journal of Heredity*, 97: 81–88.
- Magoulas, A., Tsimenides, N., and Zouros, E. 1996. Mitochondrial DNA phylogeny and the reconstruction of the population history of a species: the case of the European anchovy (*Engraulis encrasicolus*). *Molecular Biology and Evolution*, 13: 178–190.
- Mallet, J., and Barton, N. 1989. Inference from clines stabilized by frequency-dependent selection. *Genetics*, 122: 967–976.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27: 209–220.
- Minas, H. J., Codispoti, L. A., and Dugdale, R. C. 1982. Nutrients and primary production in the upwelling region off northwest Africa. *Rapports et Procès-verbaux des Réunions du Conseil International pour l'Exploration de la Mer*, 180: 141–176.
- Naciri, M., Lemaire, C., Borsa, P., and Bonhomme, F. 1999. Genetic study of the Atlantic/Mediterranean transition in sea bass (*Dicentrarchus labrax*). *Journal of Heredity*, 90: 591–596.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89: 583–590.
- Norris, R. D. 2000. Pelagic species diversity, biogeography, and evolution. *Palaeobiology*, 26: 236–258.
- Parrish, R. H., Serra, R., and Grant, W. S. 1989. The monotypic sardines, *Sardina* and *Sardinops*: their taxonomy, distribution, stock structure and zoogeography. *Canadian Journal of Fisheries and Aquatic Sciences*, 46: 2019–2036.
- Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J., and Britton-Davidian, J. 1987. Manuel de Génétique par Électrophorèses des Protéines. Collection Techniques et Documentation, Lavoisier, Paris.
- Patarnello, T., Volckaerat, F. A. M., and Catilho, R. 2007. Pillars of Hercules: is the Atlantic–Mediterranean transition a phylogeographical break? *Molecular Ecology*, 16: 4426–4444.
- Pelegrí, J. L., Aristegui, L., Cana, M., González-Dávila, A., Hernández-Guerra, S., Hernández-León, A., Marrero-Díaz, M. F., et al. 2005. Coupling between the open ocean and the coastal upwelling region off Northwest Africa: water recirculation and offshore pumping of organic matter. *Journal of Marine Systems*, 5: 3–37.
- Planes, S., and Doherty, P. J. 1997. Genetic and color interactions at a contact zone of *Acanthochromis polyacanthus*: a marine fish lacking pelagic larvae. *Evolution*, 51: 1232–1243.
- Pujolar, J. M., Roldan, M. I., and Pla, C. 2003. Genetic analysis of tuna populations, *Thunnus thynnus thynnus* and *T. alalunga*. *Marine Biology*, 143: 613–621.
- Quesada, H., Zapata, C., and Alvarez, G. 1995. A multilocus allozymes discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors. *Marine Ecology Progress Series*, 116: 99–115.
- Ramon, M. M., and Castro, J. A. 1997. Genetic variation in natural stocks of *Sardina pilchardus* (sardines) from the western Mediterranean Sea. *Heredity*, 78: 520–528.
- Raymond, M., and Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86: 248–249.
- Riginos, C., Sukhdeo, K., and Cunningham, C. W. 2002. Evidence for selection at multiple allozyme loci across a mussel hybrid zone. *Molecular Biology and Evolution*, 19: 347–351.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution*, 43: 223–225.
- Schneider, S., Roessli, D., and Excoffier, L. 2000. Arlequin Ver 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.
- Shaklee, J. B., Allendorf, F. W., Morizot, D. C., and Whitt, G. S. 1990. Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fisheries Society*, 119: 2–15.
- Sotka, E. E., and Palumbi, S. R. 2006. The use of genetic clines to estimate dispersal distances of marine larvae. *Ecology*, 87: 1094–1103.
- Sotka, E. E., Wares, J. P., Barth, J. B., Grosberg, R. K., and Palumbi, S. R. 2004. Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. *Molecular Ecology*, 13: 2143–2156.
- Stevens, I., and Johnson, J. 2003. A numerical modelling study of upwelling filaments off the NW African coast. *Oceanologica Acta*, 26: 549–564.
- Szymura, J. M., and Barton, N. H. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina orientalis* and *B. orientalis*, near Cracow in southern Poland. *Evolution*, 40: 1141–1159.
- Tudela, S., García-Marín, J. L., and Pla, C. 1999. Genetic structure of the European anchovy, *Engraulis encrasicolus* L., in the north-west Mediterranean. *Journal of Experimental Marine Biology and Ecology*, 234: 95–109.
- Ward, R. D., Woodwark, M., and Skibinski, D. O. F. 1994. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology*, 44: 213–232.
- Weir, B. S., and Cockerham, C. C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, 41: 1358–1370.
- Wright, S. 1969. *Evolution and the Genetics of Populations. 2. The Theory of Gene Frequencies*. University of Chicago Press, Chicago.