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LACK OF GENETIC DIVERGENCE BETWEEN SPAWNING AGGREGATIONS OF ANCHOVY IN THE CATALAN SEA (NORTHWESTERN MEDITERRANEAN)

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RESUM

El seitó, *Engraulis encrasicolus*, és un dels peixos pelàgics del mar Català (Mediterrània nord-occidental) amb més importància comercial. En aquest mar s'han localitzat dues àrees de posta, localitzades sota aigües jurisdiccionals franceses i espanyoles respectivament, cosa que suggeriria que el seitó del mar Català podria constituir dues subpoblacions diferenciades i, per tant, ser gestionades independentment. S'ha analitzat electroforèticament una mostra de cadascuna d'aquestes àrees de posta i no hem trobat diferències genètiques a cap dels nou *loci* polimòrfics estudiats. Tot i que aquesta informació no confirma l'existència de les dues subpoblacions discretes, altres evidències indiquen que ambdues àrees estàn parcialment aïllades. Presentem un conjunt d'hipòtesis que justificaria aquesta aparent contradicció entre les dades reproductives i genètiques.

RESUMEN

La anchoa, *Engraulis encrasicolus*, es uno de los peces pelágicos del mar Catalán (Mediterráneo noroccidental) con mayor interés comercial. En este mar, se han localizado dos áreas de puesta que se encuentran respectivamente en aguas jurisdiccionales francesas y españolas. Este hecho ha sugerido la existencia en el mar Catalán de dos subpoblaciones que podrían ser gestionadas independientemente. Se ha analizado electroforéticamente una muestra de cada una de estas áreas de puesta y no hemos encontrado diferencias genéticas en ninguno de los nueve *loci* polimórficos analizados. A pesar de que estos resultados no confirman la existencia de dos subpoblaciones discretas, otras evidencias indican que ambas áreas están parcialmente aisladas. Presentamos aquellas hipótesis que podrían justificar esta aparente contradicción entre los datos reproductivos y los genéticos.

ABSTRACT

The anchovy, *Engraulis encrasicolus*, is one of the most important pelagic commercial fish from the Catalan Sea (Northwestern Mediterranean). In this sea, two spawning areas are located under French and Spanish jurisdiction respectively, and suggest that Catalan Sea anchovies may comprise two subpopulations and therefore may be managed independently. We examined one sample from each spawning area and found no genetic differences at the nine variable protein-coding *loci* screened. Although the lack of allele-frequency differences does not confirm the existence of two genetically-discrete subpopulations, other evidence suggests the spawnning areas are at least partially isolated. A set of hypothesis that can accomodate the spawning and genetic data is presented.

Keywords: anchovy, electrophoresis, *Engraulis encrasicolus*, genetic stock identification

INTRODUCTION

The anchovy, *Engraulis encrasicolus* (L.), together with the sardine, *Sardina pilchardus* (Walbaum), are the most important pelagic commercial fishes in Spanish Mediterranean coastal waters. The anchovy is the main target of the purse-seine fleet that fishes along the Catalan coast and a large industry has been developed based on that resource (Pertierra, 1992). Anchovy landing are greatest in summer and progressively decline thereafter.

Two spawning aggregations which have been reported along the Catalan Sea showed differences in spwaning times. The Northern area, near Cape Creus, is considered to be part of a major spawning zone associated with the oceanographic features of the Gulf du Lion and the outflow of Rhône River (France), whereas the Southern area is located over the delta of Ebro River (Spain) where spawning occurs during a more extensive period from April to September (Palomeras, 1992). This fact suggested that the anchovy stock may include two subpopulations corresponding to the two spawning areas.

MATERIALS AND METHODS

This study was conducted along the Spanish Catalan coast. Three samples of anchovy were collected; one from Sant Carles de la Rapita (Southern spawning aggregation) and the other two from Port de la Selva (Northern spawning aggregation), for later electrophoretic analysis. One of the sample (PS-2) was collected in June of 1990, the other two samples (SC and PS-1) were collected before the spawning season in 1991. Samples were packed in ice for transportation to the laboratory and were stored at -60 °C until processing.

Tissue extraction, horizontal gel electrophoresis(starch 11%), buffers and procedures for visualizing proteins followed the general methodology outlined by Aebersold *et al.*(1987). Extracts from tissues including eye (E), liver (L) and skeletal muscle (M) were electrophoretically screened for resolution and activity with the buffer systems: AC (Amine citric acid buffer, pH=7.0) and TBCL (Tris boric citric lithium buffer).

Interpretable genetic patterns were obtained for eleven enzymes (Table 1). The genetic nomenclature of protein-coding loci follows the guidelines of Shaklee *et al.*(1990). The alleles were numbered from slowest to fastest according to their electrophoretic mobility from cathode to anode. The present electrophoretic analysis permitted the detection of fifteen enzyme-encoding loci (Table 1), nine of them showed genetic variation (*bGLUA**, *G3PDH**, *GPI**, *sIDHP-2**, *LDH-1**, *sMDH-1**, *sMDH-3**, *MEP**, and *PGDH**). This number of polymorphic (informative) loci is higher than the four previously used in the study of Spanakis *et al.* (1989) and similar to the observed by Bembo *et al.* (1996) in eastern Mediterranean locations. Complete loci screening was only performed with the two 1991 samples.

The statistical tests used were: Exact probability test (analogous to Fisher's exact test) in order to test the concordance of the genotypic frequencies with those expected under Hardy-Weinberg equilibrium and Chi-squared (χ^2) contingency test to evaluate the homogeneity existing among samples. Analyses were carried out using the BIOSYS-1 computer program (Swofford and Selander, 1981).

Enzyme	E.C. number	locus	buffer	tissue
Adelinate kinase	E.C.2.7.4.3	*AK**	a	M, E
Creatine kinase	E.C.2.7.3.2	*CK**	b	M, E
b-Glucosaminidase	E.C.3.2.1.30	*bGLUA**	b	L
Glycerol-3-phosphate dehydrogenase	E.C.1.1.1.8	*G3PDH**	b	M
Glucose-6-phosphate isomerase	E.C.5.3.1.9	*GPI**	a	M
Isocitrate dehydrogenase (NADP+)	E.C.1.1.1.42	*sIDHP-1**	a	M, L
		*sIDHP-2**	a	E
Lactate dehydrogenase	E.C.1.1.1.27	*LDH-1**	a	M
		*LDH-2**	a	M
Malate dehydrogenase	E.C.1.1.1.37	*sMDH-1**	a	M
		*sMDH-2**	a	M
		*sMDH-3**	a	E, L
Malic enzyme (NADP+)	E.C.1.1.1.40	*MEP**	b	M
Phosphogluconate dehydrogenase	E.C.1.1.1.44	*PGDH**	b	M
Phosphoglucomutase	E.C.5.4.2.2	*PGM**	b	M

Table 1. *Analized enzymes, Enzyme comission number, detected loci, buffers (a= AC; b=TBCL) and tissue (M= muscle; L= liver; E= eye).*

RESULTS

Allelic frequencies for variable loci are shown in Table 2. The range of genetic variability within the populations, measured as the proportion of polymorphic loci at a 5% level (common allele frequency less than or equal to 0.95), lies between 33.3% in the sample taken at Sant Carles and 26.6% of the 1991 Port de la Selva sample. The average heterozygosity per locus (H) was 0.124 and 0.096, respectively. The number of polymorphic loci (P) and the heterozygosity (H) are within the range described for eastern Mediterranean locations (Bembo *et al.*, 1996) and other clupeiforms (P= 33.4 and H= 0.071 as an average for 19 species; Hedgecock *et al.*, 1989). On the other hand, they are larger than the average values generally detected in marine teleosts (average H= 0.055 for 106 species; Smith and Fujio, 1982).

Only one out of 19 tests for concordance of observed genotypic frequencies with those expected under Hardy-Weinberg equilibrium was significant (locus *G3PDH** for 1991 Port de la Selva's sample, p=0.039). This significance was due to the presence of a homozygote for a genotype expected to occur at a low frequency and is therefore considered as a type I statistical error rather than a deviation of biological significance.

Allele-frequency differences between the two catches in Port de la Selva were

statistically non-significant for each loci at the 5% level (Table 3). According to this four-loci-comparison, we consider that there is a stability of the allele frequencies in this northern spawning aggregation during years. Therefore, geographical patterns discussed below would be stable in the long run.

Table 2. *Allelic frequencies for all variable loci for southern spawning area sample (SC) and northern spawning area samples (PS-1 and PS-2). (N= sample size).*

Locus / allele	SC	PS-1	PS-2
*bGLUA**			
N	27	35	
1	0.481	0.514	
2	0.519	0.486	
*G3PDH**			
N	28	39	40
1	0.036	0.038	0.079
2	0.964	0.962	0.921
*GPI**			
N	27	40	39
1	0.981	0.975	0.987
2	0.019	0.025	0.013
*sIDHP-2**			
N	28	40	
1	0.036	0.037	
2	0.714	0.875	
3	0.232	0.063	
4	0.018	0.025	
*LDH-1**			
N	28	39	39
1	0.714	0.718	0.654
2	0.286	0.282	0.346
*sMDH-1**			
N	28	40	39
1	0.054	-	0.013
2	0.946	1.00	0.987
*sMDH-3**			
N	28	40	
1	0.018	-	
2	0.982	1.00	
*MEP**			
N	26	38	
1	0.860	0.921	
2	0.140	0.079	
*PGDH**			
N	28	40	
1	1.00	0.987	
2	-	0.013	

Table 3. Allele-frequency heterogeneity between samples. Nloc, number of loci compared; χ^2, contingency chi-squared value; d.f. degree of freedom; prob. probability; and sig.loc. loci with significant differences.

	Nloc	χ^2	d.f.	prob.	sig.loc.
Temporal heterogeneity (PS-1 vs. PS-2)	4	3.264	4	p>0.05	none
Spatial heterogeneity (SC vs. PS-1)	9	16.237	11	p>0.05	*sIDHP-2** (p=0.040) *sMDH-1** (p=0.036)

In the chi-squared tests for heterogeneity between allelic frequencies between the two spawning aggregations (Table 3), only *sMDH-1** and *sIDHP-2** loci were significantly different. However, the sum for all the loci is not significant (χ^2 = 16.237, d.f. = 16, p > 0.05). In the case of *sMDH-1** (p=0.036) the difference is due to a excess of the rare allele in the Sant Carles' sample, while that observed for *sIDHP-2** (p=0.040) was due to variation in alleles *2 and *3. Simultaneous inference by sequential Bonferroni test (Rice, 1989) rejects the statistical significance of these two deviations at the "table-wide" 0.05 level. The general pattern is similar to that expected if the two samples had been extracted from the same panmictic population (Ryman and Stahl, 1981).

DISCUSSION

An important component of fishery management is a knowledge of the genetic population structure of the species in question (Allendorf *et al.*, 1987). This is especially important when a fishery falls under multiple political jurisdictions. Such concern is involved in the management of the anchovy ressource in the Catalan Sea, which supports the anchovy fishery of France and Spain. The 12 mile limit to national waters within the region and the absence of an "Exclusive Economic Zone" has been the cause of several conflicts between these two European Comunity members exploiting the fishery.

The biological data of Palomeras (1992) suggested the existence of two spawning aggregations of the anchovy in this sea, one located in the South under the Spanish jurisdiction and other located in the North under the French jurisdiction. Our results, however, show a lack of genetic differentiation between the two spawning aggregations. Geographic structure among *E.encrasicolus* has been associated to the existence of barriers to panmixia. In their study of Greek populations, Spanakis *et al.* (1989) observed extensive temporal variation within localities and extensive spatial fluctuations among samples within the Aegean and within the Ionian seas for two loci. Their conclusion that anchovy from this two seas forms two differentiated units was supported only by a small difference in the more stable allele fre-

quencies of the *GPI** locus. Similarly, the small genetic heterogeneity among the Adriatic sea anchovy populations and those from the Ionian was considered the result of restricted gene flow between both seas through the Strait of Otranto (Bembo *et al.*, 1996). A more genetically stable and homogeneous population structure seems to occur in the Catalan Sea, probably because the lack of major oceanographical barriers on our study area.

However, the lack of allele-frequency differentiation between localities is not sufficient evidence to dismiss the possible existence of more than one panmictic unit in the anchovy stock of the Catalan Sea. One set of hypotheses, briefly discussed below, could bring together our data with that of Palomeras (1992).

Electrophoretic effort. We recognize the preliminary nature of the present data set. The number of samples, sample sizes and number loci are small. However, in a study on *E. mordax*, with eleven polymorphic loci and sample sizes of about forthy individuals, Hedgecock *et al.*(1989), detected a complex population structure in the California central stock. Meanwhile,a wider study carried out on *E. capensis*, with sample size up to 200 individuals, revealed no significant genetic differences at ten loci between spawning areas in the South Eastern Atlantic (Grant, 1985). Therefore, if exists any degree of genetic differentiation between the spawning areas in the Catalan sea, it must be small.

Time since divergence. When the separation of two populations is too recent, not enough time has elapsed for the accumulation of genetic divergence. The two spawning areas of anchovy in the Catalan sea are under the influence of the inflows of the Rhône and Ebro rivers. These inflows are suggested to have varied during the Quaternary as a consequence of climatic fluctuacions, as well as the whole current system has (Maldonado, 1985). The last glacial event ended some 13000 y.b.p. It is therefore possible that the present spawning aggregations appeared subsequent to this date, and consequently, detectable genetic differences have not yet accumulated among them. In this situation, technologies involving analyses of mitochondrial and nuclear DNA should be applied.

Gene flow. Population genetics theory suggests that two populations can maintain genetic homegeneity when the rate of exchange is higher than one individual for generation (Wright 1943). This is true for neutral characters, as most of the protein coding loci are supposed to be. Thus, if the fidelity to return to the spawning area is small, rates of exchange between areas are high and prevent genetic differentiation.

Selective forces. The two spawning areas may represent two isolated populations with similar allele frequencies because they are under the same selective forces.

All of the above possibilities must be consider, as well as the existence of only one panmictic unit. Which are true and which are false can not be assesed with the present information and there is not doubt about the need for further investigations. Those new results will resolve more firmly the questions raised by this study and will provide a sounder biological basis for the management of the anchovy of the Catalan sea. Meanwhile, the management of the anchovy resource in the Catalan Sea needs the cooperative effort of the French and Spanish Administrations.

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