Allozyme variability in two samples of swordfish, *Xiphias gladius* L., in the North Pacific Ocean

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The swordfish, *Xiphias gladius* L., is a cosmopolitan species found in all tropical and temperate oceans (Nakamura, 1983). It is an esteemed food fish and subject to a multinational fishery throughout its range of distribution (Sakagawa, 1989). In 1991 the Pacific catch was 29,245 metric tons (t) or about 44% of the total global catch of 67,142 t (FAO, 1993).

A variety of population structures have been proposed for Pacific swordfish. From one to four stocks have been proposed on the basis of fishery data from all of the Pacific Ocean (Bartoo and Coan, 1989; Skillman, 1989; and Sosa-Nishizaki and Shimizu, 1991). Grijalva-Chon et al. (1994) examined restriction-fragment-length polymorphisms (RFLP) of mitochondrial DNA (mtDNA), and their results supported the hypothesis of one stock in the North Pacific.

Allozyme analyses have been used extensively to study population structure in fishes, especially in fishery resources, because of their relative simplicity (Utter et al., 1974, 1987; Ihssen et al., 1981). Several studies of mtDNA have demonstrated increased resolution for detecting population substructure (Ferris and Berg, 1987), and the methods used in them have complemented each other because they involve different modes of inheritance: with protein electrophoresis, phenotypic data from nuclear loci and from alleles transmitted by sexual reproduction can be analyzed, whereas with the mtDNA method, fragments of haploid DNA that are (usually) maternally inherited and evolve rapidly can be analyzed (Allendorf et al., 1987). The nuclear allelic variants involve sexual exchange among individuals, and one can infer the population fitness or reproductive success through genetic variation (Nevo, 1978). In the case of a fishery resource, fishing mortality alters the frequency of adaptative traits (Nelson and Soulé, 1987).

The intent of this study was to examine the genetic variation at protein-coding loci in swordfish in a preliminary way to test whether swordfish in two localities in the North Pacific were genetically homogeneous.

Materials and methods

Samples of white muscle, liver, and heart from 44 individuals from the N–NE Hawaiian Islands and from 50 individuals from Mexico off Baja California were used to study the allozymic variation (see Grijalva-Chon et al. [1994] for sampling and storage details).

Samples of approximately 5 g of tissue were homogenized in equal volumes of a solution consisting of 0.1 M Tris-HCl pH 8, NAD+, NADP, and polyvinylpyrrolidone (100:0.1:0.1:1, v:w:w:w), with an electric homogenizer (Tissumizer Tekmar). In the case of liver, 0.5 mL of toluene was added. Homogenized samples were centrifuged at 20,000 ×g for 20 minutes at 4°C, and supernatants transferred into capped tubes and stored at –70°C.

The allozyme analysis comprised 15 enzyme systems that encoded 26 presumptive loci (Table 1). In general, the method described by Aebersold et al. (1987) was followed, with the exception that agar was not used in the staining procedure. The support media were 12% starch gels (Sigma S4501).

The criteria of Grant and Utter (1980) and Grant et al. (1984) were followed for zymogram interpretation. Locus nomenclature followed that of Shaklee et al. (1990), which designates the loci in ascending order beginning with number 1 for the most cathodal locus. Alleles for polymorphic loci were designated by using relative electrophoretic mobilities: the most common allele at each locus was designated to be 100.

Data were analyzed with BIOSYS-1 (Swofford and Selander, 1981). A locus was considered poly-

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Table 1

Protein systems and loci resolved in North Pacific swordfish. Buffers: A = Continuous Tris-citratre II, pH 8.0 (Selander et al., 1971); B = Tris-maleate, pH 7.4 (Selander et al., 1971); C = Tris-EDTA-borate, pH 8.0 (Shaw and Prasad, 1970); D = Tris-citrate, pH 8.0 (Tracey et al., 1975); E = Aminopropyl-morpholine, pH 6.1 (Clayton and Tretiak, 1972). Stains: 1 = Shaw and Prasad (1970); 2 = Schaal and Anderson (1974); 3 = Rosa-Velez (1986). E.C.= Enzyme Commission number.

Enzyme or protein	E.C.	Locus	Tissue	Buffer	Stain
Lactate dehydrogenase	1.1.1.27	*LDH-1**	Heart	A	1
		*LDH-2**	Heart		
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-1**	Heart	A	1
		*GPI-2**	Heart		
Malic enzyme	1.1.1.40	*MEP**	Heart	A	2
Phosphoglucomutase	5.4.2.2	*PGM**	Muscle	A	2
Aspartate aminotransferase	2.6.1.1	*AAT-1**	Liver	A	2
		*AAT-2**	Liver		
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6PDH**	Heart	B	2
Xanthine dehydrogenase	1.1.1.204	*XDH**	Liver	C	2
Glutamate dehydrogenase	1.4.1.3	*GDH**	Liver	C	2
Acid phosphatase	3.1.3.2	*ACP-1**	Liver	D	1
		*ACP-2**	Liver		
Octanol dehydrogenase	1.1.1.73	*ODH**	Liver	D	1
Sorbitol dehydrogenase	1.1.1.14	*SDH-1**	Liver	D	2
		*SDH-2**	Liver		
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH**	Liver	D	2
General protein	—	*PROT-1**	Heart	D	3
		*PROT-2**	Heart		
		*PROT-3**	Muscle		
		*PROT-4**	Muscle		
Esterase	3.1.1.-	*EST**	Liver	E	1
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-1**	Heart	E	2
		*GAPDH-2**	Heart		
		*GAPDH-3**	Heart		
		*GAPDH-4**	Heart		

morphic if the frequency of the most common allele did not exceed 0.95. The mean heterozygosity over all loci was computed following Nei's (1978) unbiased formula. The Hardy-Weinberg equilibrium hypothesis was tested for all polymorphic loci by means of a chi-square (χ^2) test, by pooling genotypes when more than two alleles were observed. Lavene's correction for small sample size and Yates's correction for continuity were applied (Swofford and Selander, 1981). Exact significance probabilities were calculated only to check concordance with the χ^2 test.

Genetic distance (Nei, 1978) between localities was computed. Also, the inbreeding coefficient, F_{is}, and the standarized variance in allelic frequencies, F_{st}, were calculated to measure the amount of divergence among subpopulations (Wright, 1965). The null hypothesis $F_{is}=0$ was tested by means of $\chi^2=F_{is}{}^2n$, with $k(k-1)^2$ degrees of freedom, where k denotes number of alleles (Li and Horvitz, 1953). The null hypothesis $F_{st}=0$ was tested by means of $\chi^2=F_{st}{}^2n$, with $(s-1)(k-1)$ degrees of freedom, where s denotes the number of geographic localities and k the number of alleles (Workman and Niswander, 1970). The heterogeneity of allelic frequencies at polymorphic loci among geographic localities was tested with the log-likelihood ratio test (G-test) with Yates's correction for continuity (Zar, 1984). The number of migrants per generation was estimated by $N_em = (F_{st}{}^{-1}-1)/4$ (Hartl, 1988).

Results

Four out of twenty-six loci were polymorphic: *ODH**, *GPI-1**, *PROT-2**, and *PROT-3**. Table 2 summarizes the genetic variation fish from both localities. The average number of alleles per locus was 1.19 (Mexico), and 1.31 (Hawaii). The observed heterozygosity per locus ranged between 0.000 and 0.341 (Table 3), with a mean of 0.010 in Mexico, and 0.026 in Hawaii. The mean gene diversity for the pooled data was 0.020.

Isocitrate dehydrogenase (IDH) revealed variation in a preliminary survey. Richardson et al. (1986) showed, however, that this enzyme may mimic a genetic polymorphism where in fact it is exhibiting age-related modification. The tissues samples were held

for 1 to 1.5 years at –70°C before analysis. Because of this, IDH was eliminated from the analysis.

Only one locus was out of Hardy-Weinberg equilibrium: *GPI-1** showed heterozygote deficiency in the Mexican sample (Table 3). This result was reflected in the F_{is} value for that locus (Table 4). The genetic distance between populations was very low: it varied between 0.000 and 0.014, with a mean of 0.0007 over all loci.

Table 2

Genetic variation of North Pacific swordfish. Standard errors are in parentheses.

	Mexico	Hawaii
No. of individuals	50	44
No. of loci examined	26	26
No. of polymorphic loci	2	2
Percentage of polymorphic loci	7.69	7.69
No. of alleles per locus	1.19 (0.08)	1.31 (0.11)
Average heterozygosity		
Observed	0.010 (0.005)	0.026 (0.014)
Expected	0.013 (0.006)	0.027 (0.013)

Table 4

Genetic distance, *G*-test for allelic frequencies heterogeneity, and *F*-statistics at polymorphic loci of North Pacific swordfish. * = 0.01 < $P \leq 0.05$; ** = 0.001 < $P \leq 0.01$; and *** = $P \leq 0.001$. *D* = genetic distance; *G* = *G*-test.

	D	*G*	F_{is}	F_{st}
*ODH**	0.001	0.615	–0.054	0.026 (*)
*GPI-1**	0.000	1.837	0.338 (***)	0.008
*PROT-2**	0.002	1.489	–0.082	0.030 (*)
*PROT-3**	0.014	4.851 (*)	–0.183	0.066 (***)
Totals		8.792 (*)	–0.013	0.039 (**)

Table 3

Allelic frequencies, heterozygosity, and Hardy-Weinberg expectations of phenotypes at four polymorphic loci. Number of individuals are in brackets. Differences in sample sizes were due to inactivation of enzymes. ns= not significant; ** = $P \leq 0.01$.

					Heterozygosity			Phenotype frequencies		
Locus	Locality	Allelic frequencies			Observed	Expected	Phenotype	Observed	Expected	Chi-square
		*100	*114							
*ODH**	Mexico [39]	0.949	0.051		0.103	0.099	A	0	0.780	0.000 (ns)
							AB	4	3.844	
							B	35	35.078	
	Hawaii [42]	1.000	0.000		0.000	0.000	A	0		
							AB	0		
							B	42		
		*75	*100	*119						
*GPI-1**	Mexico [50]	0.070	0.930	0.000	0.060	0.132	B	45	43.212	9.296 (**)
							BC	3	6.576	
							C	2	0.212	
	Hawaii [41]	0.024	0.963	0.012	0.073	0.072	A	0	0.000	0.000 (ns)
							AB	1	0.975	
							AC	0	0.025	
							B	38	38.037	
							BC	2	1.951	
							C	0	0.012	
		*100	*175							
*PROT-2**	Mexico [50]	0.990	0.010		0.020	0.020	A	0	0	0.000 (ns)
							AB	1	1	
							B	49	49	
	Hawaii [42]	0.917	0.083		0.167	0.155	A	0	0.253	0.000 (ns)
							AB	7	0.494	
							B	35	35.253	
		*100	*175							
*PROT-3**	Mexico [50]	0.980	0.020		0.040	0.040	A	0	0.010	0.000 (ns)
							AB	2	1.980	
							B	48	48.010	
	Hawaii [41]	0.829	0.171		0.341	0.287	A	0	1.123	0.619 (ns)
							AB	14	11.753	
							B	27	28.123	

The analysis of heterogeneity of allelic frequencies showed relatively small but significant differences (Table 4), with the total G=8.79 (P<0.05). The weighted average of F_{is} was –0.013, not different from zero (P>0.05), and the weighted average of F_{st} was 0.039, different from zero (P<0.001). Considering the F_{st} value, the estimated number of migrants per generation was 6.2.

Discussion

North Pacific swordfish exhibit low heterozygosity. The heterozygosity in swordfish, 0.020, is low compared with the averages of 0.055 (Smith and Fujio, 1982) and 0.064 (Ward et al., 1994) reported for marine fishes. For bony fishes in general, Nevo (1978) and Winans (1980) reported 0.051 and 0.048, respectively. The Pacific blue marlin, *Makaira nigricans*, another billfish, showed a mean heterozygosity of 0.06 (Shaklee et al., 1983).

Both *PROT-2** and *PROT-3** were polymorphic in the Hawaiian population; however, the latter was responsible for the significant differences in allelic frequencies. Although *PROT-3** drove the final result in the analysis of allelic frequencies, *ODH** and *PROT-2** also contributed in a significant way to the amount of divergence as denoted by F_{st}.

The locus *IDH** showed a high number of heterozygous organisms in the Hawaiian samples during the preliminary analysis, but that result was doubtful considering the delicate nature of that enzyme (Richardson et al., 1986). Future studies should use tissues that are as fresh as possible.

The observed heterogeneity in allelic frequencies among the two samples was an unexpected result. Recently, Grijalva-Chon et al. (1994) reported no differences in genotypic frequencies in a restriction analysis of mtDNA of North Pacific swordfish and could not reject the null hypothesis of a single population. Chow (1994) carried out PCR-RFLP analysis on the control region of mtDNA and found no differences in the haplotype frequencies between western (Japan) and eastern (Baja California) Pacific specimens of swordfish.

A widespread opinion is that mtDNA analysis is a more sensitive approach for defining population structure than are allozymes (Ferris and Berg, 1987). In fact, many studies using mtDNA analysis in several species have confirmed preliminary results of stock identification or have shown differences that other methods have not detected (e.g. Avise et al., 1986; Kornfield, 1986; Hanzawa et al., 1987; Kornfield and Bogdanowicz, 1987; Ward et al., 1989).

Recently, incongruent results have been reported with mtDNA and allozymes. Ferguson et al. (1991) did not find evidence of population structure in brook char, *Salvelinus fontinalis*, using mtDNA analysis. Allozymatic variation showed, however, a significant divergence among sampled localities. Stott et al. (1992) concluded that American plaice, *Hippoglossoides platessoides*, comprise a single population in the Canadian Atlantic coast; mtDNA analysis gave less resolution than did allozymes. Also, Ward et al. (1994) reported that yellowfin tuna, *Thunnus albacares*, from the Pacific Ocean form at least two groups or populations according to allozyme analysis, comprising five polymorphic loci and contrasting with the homogeneity suggested by mtDNA restriction analysis.

Results obtained here could be explained by a recent population "bottleneck," which would have had a bigger effect on mtDNA than on nuclear genes (Ferris and Berg, 1987). However, it is very difficult to fix the time of such a bottleneck from allozyme data because, where a specific genetic distance value is obtained, the divergence time interval is very wide (Hillis and Moritz, 1990).

Another possible explanation lies in the population sex ratio. Birky et al. (1989) pointed out that extranuclear genes may show a more subdivided population than do nuclear genes, but this feature can be reversed if females are in excess. The sex ratio in our samples indicate a skew toward females (4.8:1 in samples from Mexico and 2.7:1 from Hawaii), and data from several fishing campaigns in Baja California during 1992 and 1993 indicate consistently high female:male ratios (~5.8:1, Castro-Longoria[1]). The observed sex ratio in Baja California waters, however, is not sufficiently skewed to explain the observed difference. In accordance with Birky et al. (1989), the number of females must always be more than seven per male for nuclear genes to show more subdivision than extranuclear genes.

Because the gene flow suggested by N_em was higher than the required value to prevent differentiation due to genetic drift, the third alternative explanation could be some kind of selection. In our case, the hypothesis is that selection supported a significant heterogeneity in the face of genetic flow. The proof of this hypothesis is beyond the scope of this paper. In accordance with Lewontin (1991), it is not possible to discriminate between selectionism and neutralism with static-type data such as ours.

For fishery management purposes, it is important to establish clearly the presence or absence of genetic differentiation of fishery resources among geo-

[1] Castro-Longoria, R. 1994. Universidad de Sonora, CICTUS, Rosales y Niños Héroes s/n., Hermosillo, Sonora, México. Personal commun.

graphic areas. It is widely agreed that the absence of genetic differences, as suggested by allozyme analysis, cannot be attributed to genetic identity at the DNA level (Utter et al., 1987). When differences are evident from allozyme analysis, however, the possibility of population differentiation exists to some degree. According to our results, the genetic population structure of swordfish in the North Pacific is more complicated than previously shown by Chow (1994) and Grijalva-Chon et al. (1994). From the fishery resources management point of view, it is necessary to corroborate the status of swordfish populations throughout the Pacific Ocean both with a DNA-based character set and with allozyme analysis. Moreover, future studies should include a sampling strategy that analyzes temporal variation.

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