Marine Biology (1997) 127: 359 – 367

S. Chow · H. Okamoto · Y. Uozumi · Y. Takeuchi
H. Takeyama

Genetic stock structure of the swordfish (*Xiphias gladius*) inferred by PCR-RFLP analysis of the mitochondrial DNA control region

Received: 28 August 1996 / Accepted: 2 October 1996

Abstract Restriction fragment length polymorphism (RFLP) analysis was performed on PCR amplified DNA fragments containing the control region of the swordfish (*Xiphias gladius* Linnaeus, 1758) mitochondrial DNA. A total of 456 individuals comprising 13 local samples (six Pacific, three Atlantic, the Mediterranean Sea, two Indian Ocean and the Cape of Good Hope) were surveyed with four endonucleases (*Alu* I, *Dde* I, *Hha* I and *Rsa* I), yielding a total of 52 composite genotypes. Within-sample genotypic diversity (*H*) was high ranging from 0.702 to 0.962 with a value of 0.922 for the pooled sample. Significant geographic variation in the frequencies of genotypes and restriction patterns was revealed. The Mediterranean sample was highly distinct from all other samples. Further, *Rsa* I digestion revealed high levels of polymorphism in all but the Mediterranean samples, indicating that exogenous swordfishes rarely enter that body of water. Heterogeneity between the North and South Atlantic samples was significant, both of which differed from those of the Pacific. In contrast, the Indian Ocean samples were not significantly different from the samples of South Atlantic and Pacific. Genetic differentiation among the Pacific samples was low. The results indicate that the worldwide swordfish population is genetically structured not only among, but also within ocean basins and suggest that gene flow is restricted despite the absence of geographic barriers.

Communicated by T. Ikeda, Hakodate

S. Chow (✉) · H. Okamoto · Y. Uozumi · Y. Takeuchi
National Research Institute of Far Seas Fisheries,
Shimizu, Shizuoka 424, Japan

H. Takeyama
Tokyo University of Agriculture and Technology, Koganei,
Tokyo 184, Japan

Introduction

The swordfish (*Xiphias gladius* Linnaeus, 1758) is a remarkable habitat generalist. It has a very wide range of occurrence from tropical to cold waters of all oceans, including the Mediterranean Sea, and occasionally makes feeding excursions to the bottom (deepest recorded depth = 650 m) where the temperature may be as low as 5 °C (Nakamura 1985; Carey 1989). Because of their cosmopolitan nature and the scarcity of physical barriers to prevent their migration, one might suppose the global swordfish population to be relatively homogeneous within or even among ocean basins. Yet the biological stock structure of this pelagic fish is controversial. In the Atlantic Ocean, spawning seems widespread and continuous throughout the tropical area, and larvae are found in a wide area between 26°N and 27°S (Nishikawa et al. 1985). The size composition of swordfish caught by commercial fisheries and seasonal trends are very similar on both sides of the North Atlantic (Farber 1988; Miyake and Rey 1989). On the other hand, tag and recapture data suggested north–south, but not east–west, movements in the North Atlantic (Farber 1988; Miyake and Rey 1989).

Using restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA), Magoulas et al. (1992) compared the haplotype (genotype) frequencies of swordfish samples collected in the Mediterranean, off Gibraltar (Tarifa) and in the Gulf of Guinea, and found that the Mediterranean and Tarifa samples were very similar to one another but considerably different from the Gulf of Guinea sample. However, they also noted Mediterranean swordfishes with bite marks of a dwarf shark *Isistius brasiliensis*, a species not found in the Mediterranean, suggesting that the Mediterranean swordfishes may travel to the subtropical and tropical Atlantic and return to the Mediterranean Sea. Alvarado Bremer et al. (1995), using nucleotide sequencing analysis of a short DNA fragment of the swordfish mitochondrial control region, found no

evidence of phylogeographic structuring within the Atlantic nor even between the Atlantic and Pacific. Later, however, with much larger sample sizes, Alvarado Bremer et al. (1996) reported significant heterogeneity in haplotype distributions among samples from the Mediterranean, North and South Atlantic and Pacific. Rosel and Block (1995) also sequenced the swordfish mitochondrial control region and reported heterogeneity among the Mediterranean, Atlantic and Pacific samples, but they did not consider the North and South Atlantic to be separable stocks. Despite less biological information for the Pacific swordfish, several hypotheses regarding from one to four stocks have been proposed based on the distributions of swordfish larvae and spawners, and longline catch rates (Nishikawa et al. 1985; Bartoo and Coan 1989; Sakagawa 1989; Skillman 1989; Sosa-Nishizaki and Shimizu 1991). However, mtDNA analyses supplied no evidence of genetic differentiation between samples collected from the western, central and eastern North Pacific, and suggested that there may be sufficient gene flow across the North Pacific to prevent genetic differentiation (Grijalva-Chon et al. 1994; Rosel and Block 1995; Chow 1996).

For the Atlantic and Mediterranean swordfishes, the molecular data provided by Alvarado Bremer et al. (1996) seems to support the three separate management units (the Mediterranean Sea, North and South Atlantic Ocean) proposed by the International Commission for the Conservation of Atlantic Tunas (ICCAT) (see Miyake and Rey 1989). However, the extent of population mixing and movements of fishes are still unknown. On the other hand, the mtDNA analyses of the Pacific Ocean were performed among swordfish samples collected mostly in the North Pacific Ocean (Grijalva-Chon et al. 1994; Rosel and Block 1995; Chow 1996), and none showed evidence of population structuring. Hence, collection and comparison of samples collected over a wider spatial range throughout the entire Pacific is necessary. Although highly significant differences have been reported among the Atlantic, Mediterranean and Pacific swordfish samples (Rosel and Block 1995; Alvarado Bremer et al. 1996; Chow 1996), genetic relationships among the ''stocks'' of these ocean basins are poorly understood and no swordfish sample from the Indian Ocean has been investigated so far.

We have collected swordfish samples from the Mediterranean Sea, North and South Atlantic, Indian, and North and South Pacific Oceans for mtDNA analysis. Here, we present the results of our analysis on genetic variation within and among these samples and discuss the global population structure, gene flow and movement of individuals.

Materials and methods

Collection information on the 13 swordfish samples used in this study are presented in Table 1. Muscle tissues of swordfish collected

Table 1 *Xiphias gladius*. Descriptions for the swordfish samples used in this study. Sri Lanka sample was caught by artisanal fishery around the Islands; Medit. sample was an adult or subadult caught by harpoon fishery around the Sisily Island (*AQUASTUDIO* Italy; *CICESE* Centro de Investigacion Cientifica y de Educacion Superior de Ensenada, Mexico; *BRS* Bureau of Resources Sciences, Australia; *CSIRO* Commonwealth Scientific and Industrial Research Organization, Australia; *IATTC* Inter-American Tropical Tuna Commission, USA; *IP–SP* Instituto de Pesca Sao Paulo, Brazil; *IPTP* Indo Pacific Tuna Development and Management Programme, Sri Lanka; *NIWA* National Institute of Water & Atmospheric Research Ltd. New Zealand; *NMFS* National Marine Fisheries Service, USA; *NRIFSF* National Research Institute of Far Seas Fisheries, Japan; *USC* University of South Carolina, USA; *n/a* not available)

Area, sample code	Latitude	Longitude	Date	Collaborating organization	Size range of fish	Sample size
Pacific Ocean						
Japan	24–35°N	144–154°E	November 1991–February 1992	NRIFSF	13–90 kg[a]	45
Hawaii	0–12°N	165°W	October 1994	NMFS	62–175 cm[b]	51
Mexico	24–27°N	113–115°W	January–March 1992	CICESE	89–231 cm[b]	35
Ecuador	1°N	79°W	October 1994	IATTC	90–173 cm[b]	21
Peru	8–18°S	84–124°W	June–July 1994	NRIFSF	61–186 cm[b]	53
SWPO	30–38°S	154–179°E	June 1994, July–September 1995	NRIFSF, NIWA	62–285 cm[b]	31
Indian Ocean						
SriLanka	n/a	n/a	March–April 1992	IPTP	57–144 cm[b]	28
SJava	16–17°S	118–119°E	February 1995	BRS, CSIRO	57–214 cm[b]	35
South Africa						
Cape	38°S	25°E	June 1992	NRIFSF	120–178 cm[b]	11
Atlantic Ocean						
NWA90	38–40°N	59–72°W	June–October 1990	USC	48–175 cm[c]	27
NWA93	20–30°N	57–90°W	January–April 1993	USC	120–227 cm[c]	29
Brazil	20–30°S	35–50°W	October 1994–January 1995	IP-SP	83–249 cm[b]	63
Mediterranean Sea						
Medit.	n/a	n/a	1994	AQUASTUDIO	n/a	34

[a]Dressed weight
[b]Eye to fork length
[c]Lower jaw to fork length

by foreign organizations were preserved in ethanol prior to mailing to the laboratory in Japan. Fresh or frozen tissue samples were collected by the Japanese longliners and transferred to the laboratory. Procedures for total DNA extraction from fresh, frozen or ethanol-preserved muscle tissues and PCR amplification followed by RFLP analysis are described elsewhere (Chow et al. 1993; Chow and Inoue 1993; Chow and Ushiama 1995). Primer sequences for amplifying the mtDNA segment containing the flanking tRNAs, parts of the cytochrome *b* and 12S rRNA genes and the control region are from Palumbi et al. (1991), and the nucleotide sequences are as follows: CB3R-L, 5′-CATATTAAACCCGAATG ATATTT-3′ and 12SAR-H, 5′-ATAGTGGGGTATCTAATCC-CAGTT-3′. Without further purification the amplified fragments were digested using four-base cutters, electrophoresed on a 2% agarose gel, and stained with ethidium bromide for photographing. Since preliminary analysis indicated that four endonucleases (*Alu* I, *Dde* I, *Hha* I and *Rsa* I) (New England Biolabs) revealed relatively high restriction fragment length polymorphism in the amplified fragments of the Northwest Pacific swordfish sample (Chow 1996), these four endonucleases were applied to all samples. Restriction patterns observed in each endonuclease digestion were alphabetically labeled and composited for comparing genotype frequencies between the samples. The genotypic diversity (*H*) was calculated following Nei (1987). Rogers' distance (Rogers 1972) between samples was calculated using the genotype frequencies and used for the UPGMA (unweighted pair-group method using an arithmetic average) cluster analysis (Sneath and Sokal 1973). Chi-square analysis was conducted using the Monte Carlo simulation of Roff and Bentzen (1989) with 1000 randomizations of the data to test heterogeneity of the frequency distributions of the genotypes between and among samples.

Results

A single fragment of approximately 1900 base pairs was amplified in all individuals and no apparent size difference among individuals was observed. Restriction profiles observed by each of the four endonucleases are shown in Fig. 1. Four restriction patterns were observed in *Alu* I and *Hha* I, seven in *Dde* I, and eight in *Rsa* I. The frequencies of each restriction pattern are presented in Table 2. Most samples shared the most common restriction pattern in each endonuclease digestion, while the frequencies of restriction patterns varied among the samples. Pattern *C* in *Alu* I digestion was the most common in the northwestern Atlantic samples (NWA90 and NWA93), while Pattern *A* prevailed in the other samples. Pattern *C* in *Dde* I and *B* in *Hha* I digestions

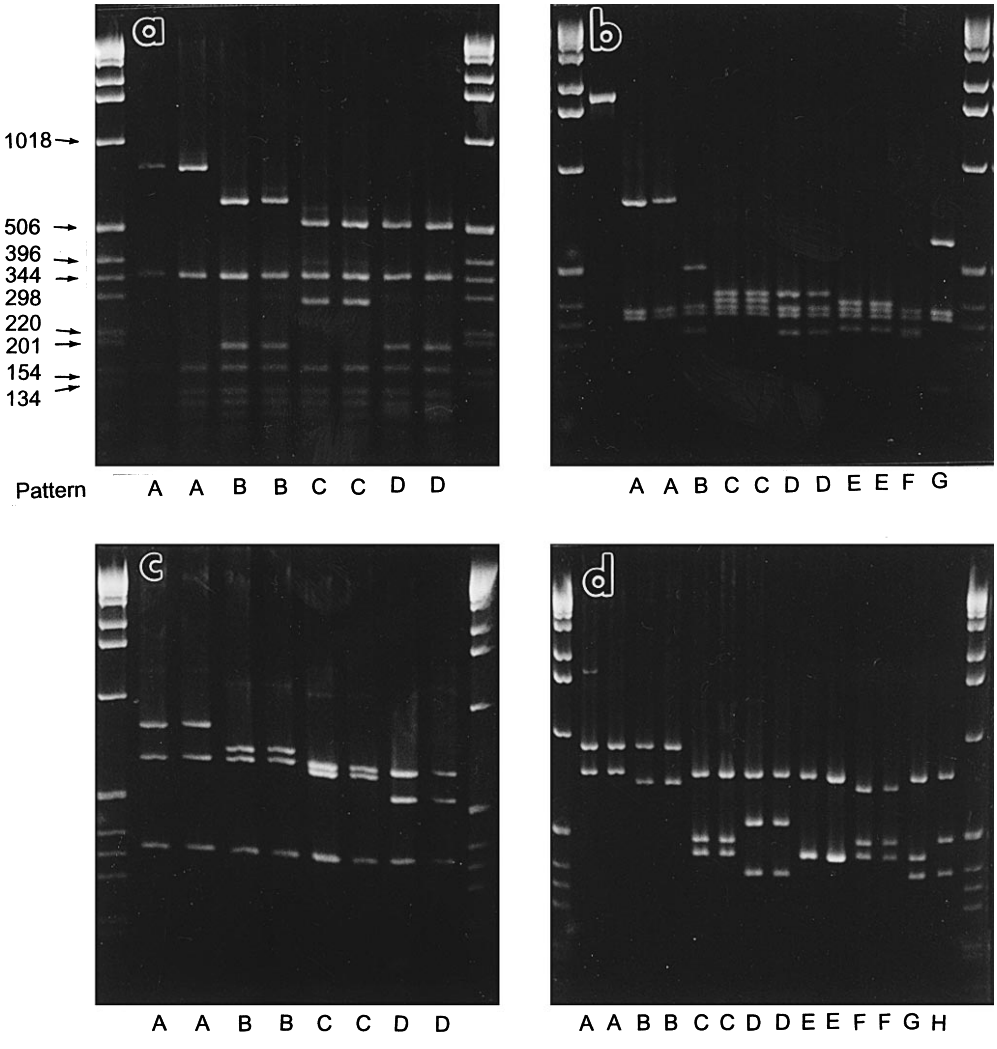

Fig. 1 *Xiphias gladius*. Gel electrophoresis of 1900 bp DNA fragment containing mitochondrial control region digested by **a** *Alu* I, **b** *Dde* I, **c** *Hha* I and **d** *Rsa* I, showing restriction fragment length polymorphisms. Left and right ends are 1 kb DNA ladder (GIBCO, BRL) and sizes (in base pairs) are indicated along left margin of **a.** Alphabetic nomenclatures on each restriction pattern are shown at the bottom of each photo. Undigested fragment was loaded in the second to left lane of **b**

Table 2 *Xiphias gladius*. Frequencies of the restriction patterns of 13 local samples

	Japan	Hawaii	Mexico	Ecuador	Peru	SWPO	SriLanka	SJava	Cape	Brazil	NWA90	NWA93	Medit.
Alu I													
A	0.578	0.588	0.6	0.619	0.641	0.612	0.571	0.629	0.636	0.508	0.296	0.276	0.53
B	0.2	0.157	0.286	0.048	0.151	0.194	0.143	0.114	0.091	0.051	0.037	0.103	0.029
C	0.2	0.255	0.114	0.333	0.17	0.194	0.179	0.257	0.273	0.424	0.556	0.621	0.412
D	0.022	0	0	0	0.038	0	0.107	0	0	0.017	0.111	0	0.029
(*n*)	(45)	(51)	(35)	(21)	(53)	(31)	(28)	(35)	(11)	(59)	(27)	(29)	(34)
Dde I													
A	0.156	0.059	0.176	0.143	0.226	0.194	0.148	0.057	0.091	0.065	0.111	0	0
B	0.022	0	0	0	0	0	0	0.029	0	0	0	0	0
C	0.733	0.745	0.765	0.714	0.736	0.774	0.667	0.686	0.909	0.758	0.852	0.897	0.794
D	0.089	0.176	0.059	0.095	0.038	0.032	0.185	0.229	0	0.129	0	0.103	0.206
E	0	0.02	0	0	0	0	0	0	0	0.032	0.037	0	0
F	0	0	0	0	0	0	0	0	0	0.016	0	0	0
G	0	0	0	0.048	0	0	0	0	0	0	0	0	0
(*n*)	(45)	(51)	(34)	(21)	(53)	(31)	(27)	(35)	(11)	(62)	(27)	(29)	(34)
Hha I													
A	0.222	0.078	0.118	0.238	0.189	0.226	0.107	0.057	0	0	0.071	0.034	0
B	0.733	0.863	0.882	0.667	0.811	0.774	0.893	0.914	1	0.968	0.857	0.932	1
C	0.045	0.059	0	0.095	0	0	0	0.029	0	0.016	0.036	0.034	0
D	0	0	0	0	0	0	0	0	0	0.016	0.036	0	0
(*n*)	(45)	(51)	(34)	(21)	(53)	(31)	(28)	(35)	(11)	(62)	(28)	(29)	(34)
Rsa I													
A	0.378	0.392	0.429	0.333	0.358	0.484	0.285	0.457	0.273	0.318	0.148	0.242	0
B	0.067	0.118	0.085	0.095	0.038	0.065	0	0.029	0.091	0.031	0	0.069	0
C	0.511	0.392	0.429	0.286	0.377	0.322	0.607	0.400	0.545	0.508	0.778	0.621	1
D	0	0.039	0.057	0.143	0.076	0.065	0	0.029	0	0.048	0	0	0
E	0.022	0.059	0	0.095	0.132	0.032	0.036	0.085	0.091	0.079	0.074	0.034	0
F	0.022	0	0	0.048	0.019	0.032	0.036	0	0	0.016	0	0	0
G	0	0	0	0	0	0	0	0	0	0	0	0.034	0
H	0	0	0	0	0	0	0.036	0	0	0	0	0	0
(*n*)	(45)	(51)	(35)	(21)	(53)	(31)	(28)	(35)	(11)	(63)	(27)	(29)	(34)

were the most frequent in all samples. The most commonly observed pattern in *Rsa* I was *A* in the Ecuador, southwestern Pacific (SWPO) and South Java (SJava) samples but *C* in the others. *Rsa* I digestion revealed very high levels of polymorphism in all but the Mediterranean (Medit.) samples.

Compositing the restriction patterns of the four endonucleases yielded a total of 52 genotypes among 456 individuals analyzed in the 13 samples (Table 3). The genotypic diversity (*H*) was very high with a value of 0.922 for the pooled sample, and varied from 0.702 (Medit.) to 0.962 (Ecuador). Medit., NWA90 and

Table 3 *Xiphias gladius*. Frequency distribution of 52 genotypes and genotypic diversity (*H*) in 13 swordfish samples. Letters in genotypes denote restriction patterns for *Alu* I, *Dde* I, *Hha* I and *Rsa* I from left to right

Genotype	Japan	Hawaii	Mexico	Ecuador	Peru	SWPO	Sri-Lanka	SJava	Cape	Brazil	NWA90	NWA93	Medit.	Total
AAAA	3	1	3	2	6	5	0	0	0	0	0	0	0	20
AAAC	2	1	0	0	2	0	2	1	0	0	1	0	0	9
AABC	0	0	3	0	2	0	2	0	1	4	1	0	0	13
AABE	1	1	0	1	1	1	0	1	0	0	0	0	0	6
ABBA	1	0	0	0	0	0	0	1	0	0	0	0	0	2
ACAA	2	2	1	1	0	1	0	1	0	0	1	0	0	9
ACAC	3	0	0	1	2	1	1	0	0	0	0	0	0	8
ACBA	1	2	3	2	3	3	1	3	2	10	1	3	0	34
ACBB	2	2	2	0	2	2	0	0	0	0	0	0	0	10
ACBC	6	13	7	3	11	5	3	8	3	9	3	5	11	87
ACBE	0	2	0	1	3	0	0	1	1	1	0	0	0	9
ACBF	1	0	0	0	1	1	1	0	0	1	0	0	0	5
ACCB	0	0	0	1	0	0	0	0	0	0	0	0	0	1
ADBA	3	2	1	0	0	0	3	3	0	2	0	0	0	14
ADBC	1	2	0	0	1	0	2	2	0	2	0	0	7	17
ADCA	0	1	0	0	0	0	0	0	0	0	0	0	0	1
ADCD	0	0	0	0	0	0	0	1	0	0	0	0	0	1
AEBA	0	1	0	0	0	0	0	0	0	0	0	0	0	1
AFBC	0	0	0	0	0	0	0	0	0	1	0	0	0	1
AGAA	0	0	0	1	0	0	0	0	0	0	0	0	0	1
BABE	0	0	0	0	1	0	0	0	0	0	0	0	0	1
BCAA	0	0	0	0	0	0	0	0	0	0	0	1	0	1
BCBA	3	6	4	0	6	2	1	3	1	2	1	2	0	31
BCBB	1	0	0	0	0	0	0	0	0	0	0	0	0	1
BCBC	5	1	4	1	0	3	3	0	0	1	0	0	1	19
BCBD	0	0	2	0	0	0	0	0	0	0	0	0	0	2
BCBE	0	0	0	0	1	0	0	0	0	0	0	0	0	1
BDBA	0	1	0	0	0	1	0	1	0	0	0	0	0	3
CACA	1	0	0	0	0	0	0	0	0	0	0	0	0	1
CCBA	1	3	2	0	3	3	2	4	0	4	0	0	0	22
CCBB	0	1	0	0	0	0	0	0	1	0	0	0	0	2
CCBC	6	3	1	1	2	1	1	3	2	9	10	12	14	65
CCBD	0	2	0	3	2	2	0	0	0	3	0	0	0	12
CCBE	0	0	0	0	1	0	1	1	0	3	2	1	0	9
CCBG	0	0	0	0	0	0	0	0	0	0	0	1	0	1
CCBH	0	0	0	0	0	0	1	0	0	0	0	0	0	2
CCCA	1	1	0	1	0	0	0	0	0	0	1	0	0	4
CCCC	0	0	0	0	0	0	0	0	0	1	0	1	0	2
CCDC	0	0	0	0	0	0	0	0	0	0	1	0	0	1
CDBA	0	0	0	0	1	0	0	0	0	0	0	1	0	2
CDBB	0	2	1	1	0	0	0	1	0	1	0	2	0	8
CDBC	0	0	0	0	0	0	0	0	0	1	0	0	0	1
CDBF	0	0	0	1	0	0	0	0	0	0	0	0	0	1
CDCB	0	1	0	0	0	0	0	0	0	0	0	0	0	1
CDDA	0	0	0	0	0	0	0	0	0	1	0	0	0	1
CEBC	0	0	0	0	0	0	0	0	0	0	1	0	0	1
CEBE	0	0	0	0	0	0	0	0	0	1	0	0	0	1
CFBC	0	0	0	0	0	0	0	0	0	1	0	0	0	1
DCBA	1	0	0	0	0	0	1	0	0	0	0	0	0	2
DCBC	0	0	0	0	0	0	2	0	0	0	3	0	1	6
DCBD	0	0	0	0	2	0	0	0	0	0	0	0	0	2
DDBB	0	0	0	0	0	0	0	0	0	1	0	0	0	1
No. individual	45	51	34	21	53	31	27	35	11	59	26	29	34	456
H	0.944	0.916	0.920	0.962	0.927	0.931	0.960	0.921	0.909	0.919	0.840	0.801	0.702	0.922

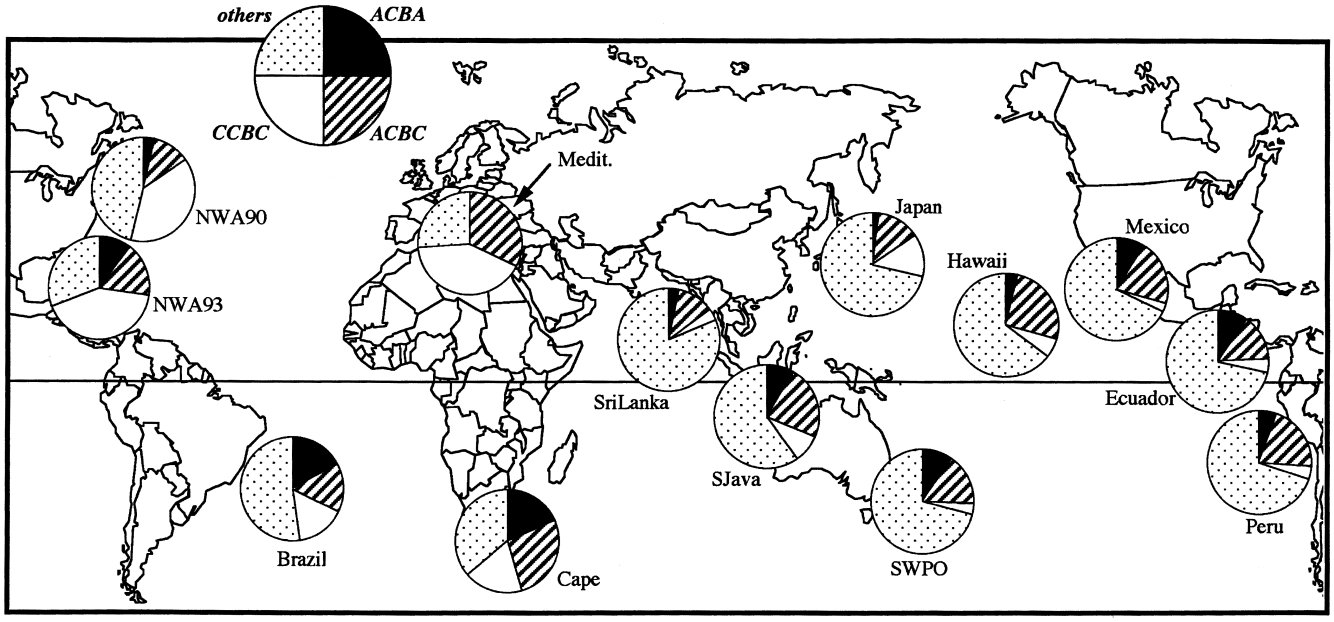

Fig. 2 *Xiphias gladius.* Pie-graph representation for the genotype frequencies of 13 local samples. Three most common genotypes (*ACBA*, *ACBC* and *CCBC*) were selected and the other genotypes were pooled

NWA93 were less variable (H ranged from 0.702 to 0.84) than the others (0.909 to 0.962). For pie-graph representation of genotype frequencies, the first to third most common genotypes (*ACBA*, *ACBC* and *CCBC*) were selected and the other genotypes were pooled (Fig. 2). Approximately 40% of the genotypes was represented by the *CCBC* in the Mediterranean and North Atlantic samples, and this may be responsible for their lower genotypic diversity. In all Pacific, SriLanka, and SJava samples, the *CCBC* genotype was much less frequent (3 to 13%) and genotype *ACBC* was most common. The *CCBC* genotype was also less frequent in the Brazil and Cape samples (15 and 18%, respectively). The Brazil and Cape samples had a characteristic higher frequency of the genotype *ACBA* (16.9 and 18.2%, respectively) than the others (0 to 10.3%). A UPGMA phenogram based on the Rogers' distances calculated from the genotype frequencies is shown in Fig. 3. Two major clusters were obvious in the phenogram, one of which contained the Medit., NWA90 and NWA93 samples, and the other all remaining samples. Among the Pacific samples, there was no correlation between geographic relationships and position in the phenogram.

Results of the heterogeneity test performed on the genotype frequencies among samples are shown in Table 4. The Cape sample was not included in this analysis because of its small size ($n = 11$). Chi-square analysis among the remaining 12 samples was highly significant ($P < 0.0001$). Chi-square analysis among samples excluding the Medit. sample and the Medit. and NWA samples still showed high heterogeneity ($P < 0.0001$ and $P = 0.030$, respectively). In contrast, no significant heterogeneity was observed among the Pacific samples ($P = 0.325$). Chi-square analysis was not significant even when the SriLanka and SJava samples were added to the Pacific samples, but the probability was much smaller ($P = 0.091$). Highly significant heterogeneity was observed among the Mediterranean and Atlantic samples (Medit., NWA90, NWA93 and Brazil) ($P = 0.001$). Although the Pacific samples were collected

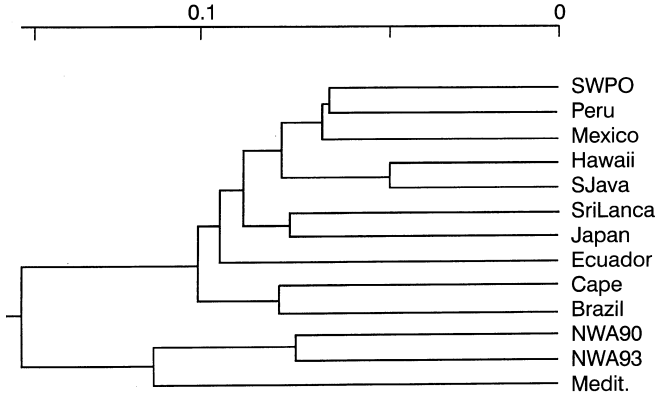

Fig. 3 *Xiphias gladius.* UPGMA phenogram for 13 local samples

Table 4 *Xiphias gladius.* Results of chi-square analysis of heterogeneity on the distributions of genotype frequencies among samples. Cape sample was not included because of its small size ($n = 11$)

Samples	n	χ^2	P
Total	12	714.934	<0.0001
w/o Medit.	11	616.145	<0.0001
w/o Medit. and NWA	9	418.060	0.030
Pacific, Sri Lanka, SJava	8	305.672	0.091
Pacific	6	191.698	0.325
Medit., NWA and Brazil	4	123.320	0.001

Table 5 *Xiphias gladius*. Results of chi-square analysis for pairwise sample comparisons. Several samples were pooled to avoid the effects of large numbers of tests, creating the Pacific (all six Pacific samples were pooled), the Indian (SriLanka and SJava samples), the South Atlantic (Brazil and Cape samples) and the northwestern Atlantic (NWA90 and NWA93 samples) samples. Significant levels were $*0.0001 \leq P < 0.005$ and $**P < 0.0001$

	Pacific	Indian	South Atlantic	NWA
Pacific				
Indian	51.689			
South Atlantic	75.763*	36.990		
NWA	114.583**	49.905*	43.29*	
Mediterranean	94.047**	41.031**	40.582**	29.965*

throughout a much wider range than in the Atlantic and Mediterranean, much less heterogeneity among the Pacific samples was observed despite the higher level of within-sample variation.

Chi-square analysis was also performed for pairwise sample comparisons. Several samples were pooled to avoid the effects of large numbers of tests, creating the Pacific (all six Pacific samples were pooled), the Indian (SriLanka and SJava samples), the South Atlantic (Brazil and Cape samples) and the northwestern Atlantic (NWA90 and NWA93 samples) samples. This manipulation may be justified by the results of chi-square analysis among samples, cluster analysis and geographic assortment. The results are shown in Table 5, where the probabilities smaller than 0.005 are defined as significant after Bonferroni correction for multiple comparison (see Jobson 1991, pp 410–411). Mediterranean and northwestern Atlantic samples significantly differed from one another and also from all the other samples. While the Indian sample showed no significant differences from the South Atlantic or Pacific samples, the difference between the South Atlantic and Pacific samples was significant.

Discussion

The present study clearly indicates that the swordfish population is genetically structured not only among but in some cases also within ocean basins. Based on the results from the cluster analysis and the heterogeneity test, it may be proposed that the swordfish population consists of at least four groups (stocks). The frequency distributions of restriction patterns and genotypes between the samples from the contiguous localities may imply that these groups are hierarchically arranged, with the Mediterranean and Pacific groups being the two extremes; the North Atlantic group has affinity with the Mediterranean group, and the South Atlantic group falls between the North Atlantic and Indo-Pacific groups.

Magoulas et al. (1992), Rosel and Block (1995), Alvarado Bremer et al. (1996) and Chow (1996) all have found very significant genetic differentiation among samples from the Mediterranean Sea, Atlantic and Pacific Oceans, but none of them could present evidence of complete genetic isolation among samples from these ocean basins. Our finding of very high polymorphism obtained by *Rsa* I digestion in all but the Mediterranean samples indicates that exogenous swordfishes rarely enter this sea. Although this indicates that the Mediterranean swordfishes form a genetically distinct stock, it is possible that they may leave this body of water and occasionally be caught with their exogenous counterpart in the Atlantic Ocean. Our PCR-RFLP analysis and the nucleotide sequence analysis performed by Alvarado Bremer et al. (1996) both indicated that the Mediterranean sample was closer to the North than to the South Atlantic sample, implying distance-based genetic relationships between stocks. Based on temporal variation in the gonadal index of female swordfish, de la Serna et al. (1992) suggested movements of swordfish into the Mediterranean Sea from the Gibraltar area. However, Magoulas et al. (1992) reported that the mtDNA haplotype distributions of the sample from Tarifa (at the mouth of Gibraltar) were very similar to those from the Mediterranean Sea but considerably different from the Gulf of Guinea sample. Alvarado Bremer et al. (1996) (and their personal communication) observed that their northeastern Atlantic sample (20 to 40°N; 10 to 15°W) had very similar haplotype distributions to their northwestern samples (the Grand Banks and Gulf of Mexico), but these were significantly different from the sample from the Gibraltar area which was almost identical with that of the Mediterranean. These findings suggest that many swordfishes in the Gibraltar area are actually of Mediterranean origin and repeatedly enter and leave this sea. However, the extent of migration of those leaving the Mediterranean seems to be limited. Thus, we conclude that the genetic isolation between Mediterranean swordfish and others is nearly complete.

In the North Atlantic, a single stock hypothesis has currently been adopted by the ICCAT for managing the swordfish fishery, but a two-stock hypothesis (separating west and east) is also considered (Miyake and Rey 1989). North–south, but no substantial east–west, movements have been suggested by tag and recapture data (Farber 1988), supporting the two-stock hypothesis in the North Atlantic. Recently, however, one individual released in the northwestern Atlantic (38°N; 73°W) was recaptured in the east (37°N; 15°W) (Dr. J. Mejuto personal communication). Although at present there is no concrete evidence on this matter, it is possible that the temporal expansion of the swordfishes ''leaked'' from the Mediterranean into the North Atlantic water may affect the biological assessment.

Significant heterogeneity between the North and South Atlantic samples coincides with the results obtained by Alvarado Bremer et al. (1996). It is likely that the genetic homogeneity within the entire Atlantic proposed by Rosel and Block (1995) is due to their small sample size. So far, no swordfish released in the North Atlantic has been recaptured in the South Atlantic (Dr. Z. Suzuki personal communication). Although not discussed by Alvarado Bremer et al. (1996), the genotype

frequency distribution of their South Atlantic sample seemed to be intermediate between their North Atlantic and Pacific samples, a result very similar to the present study. It is possible that there is some genetic exchange between the stocks to which these samples belong and that our Brazil and Cape samples contain individuals travelling from the Indo-Pacific and/or the North Atlantic. ICCAT considers that an isolated stock exists in the South Atlantic separate from the North Atlantic, whereas swordfish concentrations off the tip of South Africa suggest some exchange of fish between the Indian and Atlantic Oceans (Miyake and Rey 1989). Indeed, our analysis showed no significant difference between the South Atlantic and Indian samples, supporting the above idea. The spawning of swordfish in the Indian Ocean appears to be concentrated in the eastern half of that ocean (Nishikawa et al. 1985). Trueborn swordfishes of the Indian Ocean may make feeding migrations to the waters around South Africa and sometimes penetrate into the Atlantic Ocean, and our data does not preclude the presence of extensive mixing between stocks of the South Atlantic and Indian Oceans. Although not significant, the genotype distribution of the South Atlantic sample is somewhat different from that of the Indian Ocean. Especially, the frequency of the *ACBA* genotype is uniquely higher in the Brazil and Cape samples than in the others, suggesting that there may be a genetically distinct unit in the South Atlantic from contiguous groups. Extensive mixing between South Atlantic and Indian Ocean swordfishes is highly possible, but the genetic contribution of the Indo-Pacific swordfishes to the South Atlantic group seems to be restricted.

The small degree of genetic differentiation among the samples of the Pacific swordfish observed in the present study highly contrasts with the population structuring observed in the Atlantic, and reconfirms the previous genetic analyses on the Pacific swordfish (Grijalva-Chon et al. 1994; Rosel and Block 1995; Chow 1996). Since gene frequency data alone may not reveal the amount of gene flow (Slatkin 1987), the above data do not necessarily indicate the presence of extensive gene flow throughout the Pacific. Nothing is known of differential oceanic structures between the Atlantic and Pacific Oceans which may prevent gene flow between the swordfish populations of the northern and southern hemispheres in the Atlantic but not in the Pacific. Despite their generalist nature for habitat selection and dispersal capability, swordfish might be less migratory than previously thought.

The family Xiphiidae is phylogenetically new, having no fossil record before the Pliocene. All fossils have been found in the Atlantic and Mediterranean (Fierstine 1989), suggesting that swordfish arose in these areas. Given swordfish immigration into the Pacific realm to be geologically very recent, there might have been insufficient time for rearrangements in the mitochondrial genotype between existing "stocks". Therefore, the mtDNA marker might not be useful for clarifying the genetic population structure of the Pacific swordfish. Since direct nucleotide sequencing of targeted, highly polymorphic DNA segments has been thought to be a very powerful strategy for examining population structure, several researchers have applied the sequencing method to the swordfish mitochondrial control region (Finnerty and Block 1992; Alvarado Bremer et al. 1995; Rosel and Block 1995; Alvarado Bremer et al. 1996). These studies proved the swordfish control region to be hypervariable. However, the creation of many new but rare genotypes did not greatly increase resolving power and might even have obscured existing differences between stocks, because the sampling variation inherent in characterizing large numbers of characters on small samples may overwhelm the variation resulting from stock divergence (Epifanio et al. 1995). In fact, Rosel and Block (1995) recommended the use of a less variable sequence than the control region for more intensive investigation of the genetic population structure in this species. The PCR-RFLP analysis adopted in the present study may be more suited for investigating swordfish population structure. It is essential to choose a subset of restriction endonucleases that optimizes genetic divergence, relative to the within-population sampling variation (Epifanio et al. 1995). The RFLP analysis actually allowed Alvarado Bremer et al. (1996) to double the sample size and to decrease the number of genotypes, consequently detecting significant differences between the North and South Atlantic swordfish samples and Atlantic and Pacific samples.

No matter what analytical method is adopted, mtDNA data alone can not provide evidence on population mixing because of the haploid nature. For example, Chow and Ushiama (1995) observed large genetic differentiation between the Atlantic and Pacific albacore (*Thunnus alalunga*) samples using the PCR-RFLP analysis on the mitochondrial ATPase gene. They observed that the albacore sample from the Cape of Good Hope had intermediate genotype frequencies between those of the Atlantic and Pacific, suggesting occurrence of population mixing. Therefore, they emphasized the importance of analyzing the nuclear genome to clarify population mixing. Investigation and utilization of highly polymorphic nuclear gene markers is necessary for further analysis of swordfish population structure. Nevertheless, the mtDNA gene marker investigated in the present study might be useful for monitoring the extent and dynamics of population mixing, especially in the Atlantic and Mediterranean, if continuous spatio-temporal sampling of swordfish is realized.

Acknowledgements We gratefully acknowledge J. R. Alvarado-Bremer and R. D. Ward for their invaluable comments and suggestions on an earlier draft of this manuscript. We would like to thank the following people for their time and effort collecting swordfish samples: J. R. Alvarado-Bremer and B. Ely, the University of South Carolina; A. F. Amorim, the Institute de Pesca, Brazil; A. Caton, Bureau of Resources Sciences, Australia; A. Di Natale, AQUASTUDIO, Italy; G. De Metrio, the University of Bari, Italy; P. Grewe, Commonwealth Scientific and Industrial Research Organization, Australia; L. Griggs, the National Institute

of Water & Atmospheric Research, New Zealand; M. G. Hinton, J. Ganchozo, E. Largacha and J. Parraga, the Inter-American Tropical Tuna Commission, USA; S. Kalish, the Ministry of Agriculture and Fishery, New Zealand; O. Sosa-Nishizaki and J. M. Grijalva-Chon, Centro de Investigacion Cientifica y de Education Superior de Ensenada, Mexico; J. Uchiyama, the National Marine Fisheries Service; and M. Yesaki, the Indo-Pacific Tuna Development and Management Program, Sri Lanka. Thanks are also due to I. Warashina, Y. Tanaka, Y. Nishikawa, Z. Suzuki, and H. Nakano, the National Research Institute of Far Seas Fisheries (NRIFSF), for assisting with sample collection and for reading the manuscript, and M. Goto, the Institute of Cetacean Research for helping with the statistical analysis.

References

Alvarado-Bremer JR, Baker AJ, Mejuto J (1995) Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (*Xiphias gladius*) populations in the Atlantic Ocean. Can J Fish aquat Sciences 52: 1720–1732

Alvarado-Bremer JR, Mejuto J, Greig TW, Ely B (1996) Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. J exp mar Biol Ecol 197: 295–310

Bartoo NW, Coan LJ (1989) An assessment of the Pacific swordfish resource. In: Stroud RH (ed) Planning the future of billfishes, research and management in the 90's and beyond. Proc 2nd int Billfish Symp, Kailua-Kona, Hawaii, 1–5 August 1988. Marine Recreational Fisheries 13, National Coalition for Marine Conservation, Inc., Savannah, Ga., pp 137–151

Carey FG (1989) Further acoustic telemetry observations of swordfish. In: Stroud RH (ed) Planning the future of billfishes, research and management in the 90's and beyond. Proc 2nd int Billfish Symp, Kailua-Kona, Hawaii, 1–5 August 1988. Marine Recreational Fisheries 13, National Coalition for Marine Conservation, Inc., Savannah, Ga., pp 103–122

Chow S (1996) Genetic comparison of Pacific and Mediterranean swordfish (*Xiphias gladius*) by RFLP analysis of the mitochondrial D-loop region. Fish Bull, US (in press)

Chow S, Clarke ME, Walsh PJ (1993) PCR-RFLP analysis on thirteen western Atlantic snappers (subfamily Lutjaninae): a simple method for species and stock identification. Fish Bull, US 91: 619–627

Chow S, Inoue S (1993) Intra- and interspecific restriction fragment length polymorphism in mitochondrial genes of *Thunnus* tuna species. Bull natn Res Inst Far Seas Fish 30: 207–225

Chow S, Ushiama H (1995) Global population structure of albacore (*Thunnus alalunga*) inferred by RFLP analysis of the mitochondrial ATPase gene. Mar Biol 123: 39–45

de la Serna JM, Alot E, Godoy MD (1992) Analysis preliminar de la madurez sexual del pez espada (*Xiphias gladius*) en el area Atlantica proxima al estrecho de Gibraltar. ICCAT, Coll Vol Sci Pap XXXIX: 522–537

Epifanio JM, Smouse PE, Kobak CJ, Brown BL (1995) Mitochondrial DNA divergence among populations of American shad (*Alosa sapidissima*): how much variation is enough for mixed-stock analysis? Can J Fish aquat Sciences 52: 1688–1702

Farber MI (1988) An overview of available information pertinent to interpreting possible stock structure of swordfish in the Atlantic Ocean. ICCAT, Coll Vol Sci Pap XXVII: 240–255

Fierstine HL (1989) A paleontological review of three billfish families (Istiophoridae, Xiphiidae, and Xiphiorhynchidae). In: Stroud RH (ed) Planning the future of billfishes, research and management in the 90's and beyond. Proc 2nd int Billfish Symp, Kailua-Kona, Hawaii, 1–5 August 1988. Marine Recreational Fisheries 13, National Coalition for Marine Conservation, Inc., Savannah, Ga., pp 11–19

Finnerty JR, Block BA (1992) Direct sequencing of mitochondrial DNA detects highly divergent haplotypes in blue marlin (*Makaira nigricans*). Molec mar Biol Biotechnol 1: 1–9

Grijalva-Chon JM, Numachi K, Sosa-Nishizaki O, de la Rosa-Velez J (1994) Mitochondrial DNA analysis of North Pacific swordfish (*Xiphias gladius*) population structure. Mar Ecol Prog Ser 115: 15–19

Jobson JD (1991) Applied multivariate data analysis. Vol. I. Regression and experimental design. Springer-Verlag, New York

Magoulas A, Kotoulas G, de la Serna JM, De Metrio G, Tsimenides N, Zouros E (1992) Genetic structure of swordfish (*Xiphias gladius*) populations of the Mediterranean and the eastern side of the Atlantic: analysis by mitochondrial DNA markers. ICCAT, Coll Vol Sci Pap XL: 126–136

Miyake P, Rey JC (1989) Status of Atlantic broadbill swordfish stocks. In: Stroud RH (ed) Planning the future of billfishes, research and management in the 90's and beyond. Proc 2nd int Billfish Symp, Kailua-Kona, Hawaii, 1–5 August 1988. Marine Recreational Fisheries 13, National Coalition for Marine Conservation, Inc., Savannah, Ga., pp 115–136

Nakamura I (1985) FAO species catalogue. Vol. 5. Billfishes of the world: an annotated and illustrated catalogue of marlins, sailfishes, spearfishes and swordfishes known to date. FAO Fish Synopsis 125: 1–65

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Nishikawa Y, Honma M, Ueyanagi S, Kikawa S (1985) Average distribution of larvae of oceanic species of scombrid fishes, 1956–1981. Far Seas Fisheries Research Laboratory, Shimizu

Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR. University of Hawaii, Honolulu

Roff DA, Bentzen P (1989) The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small sample sizes. Molec Biol Evolut 6: 539–545

Rogers JS (1972) Measures of genetic similarity and genetic distance. In: Studies in genetics. VII. University of Texas, Austin

Rosel PE, Block BA (1995) Mitochondrial control region variability and global population structure in the swordfish, *Xiphias gladius*. Mar Biol 125: 11–22

Sakagawa G (1989) Trends in fisheries for swordfish in the Pacific Ocean. In: Stroud RH (ed) Planning the future of billfishes, research and management in the 90's and beyond. Proc 2nd int Billfish Symp, Kailua-Kona, Hawaii, 1–5 August 1988. Marine Recreational Fisheries 13, National Coalition for Marine Conservation, Inc., Savannah, Ga., pp 61–79

Skillman RA (1989) Status of Pacific billfish stock. In: Stroud RH (ed) Planning the future of billfishes, research and management in the 90's and beyond. Proc 2nd int Billfish Symp, Kailua-Kona, Hawaii, 1–5 August 1988. Marine Recreational Fisheries 13, National Coalition for Marine Conservation, Inc., Savannah, Ga., pp 179–195

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science, NY 236: 787–792

Sneath PHA, Sokal RR (1973) Numerical taxonomy. Freeman, San Francisco

Sosa-Nishizaki O, Shimizu M (1991) Spatial and temporal CPUE trends and stock unit inferred from them for the Pacific swordfish caught by the Japanese tuna longline fishery. Bull natn Res Inst Far Seas Fish 28: 75–89