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Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish

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Variants in the calmodulin gene intron 4 (*CaM*) and the mtDNA D-loop region (*D-loop*) detected by RFLP and nucleotide sequence analyses were used to investigate the global stock structure of the swordfish *Xiphias gladius*. Two alleles (*A* and *B*) were observed at the *CaM* locus among 8 samples (Mediterranean, Tarifa, four Atlantic areas, Indian and Pacific oceans) comprising 567 individuals. Genotype distributions at this locus within samples were in accordance with Hardy–Weinberg equilibrium. Significant differences in allele frequencies were observed between Mediterranean–Northwest Atlantic samples (A=0·347–0·493) and those from tropical Atlantic and Indo–Pacific (A=0·81–1). In the *D-loop* region, *Alu* I and *Rsa* I digestions detected 4 (*A–D*) and 8 (*A–H*) genotypes, respectively. The frequency of type *C* for *Alu* I was lower in Indo-Pacific samples (0·2) than Atlantic and Mediterranean samples (0·365–0·643). Frequency of type *C* for *Rsa* I increased from the Pacific (0·398) to the Northwest Atlantic (0·698) and Tarifa (0·949) samples, and the Mediterranean sample was fixed for type *C*. These samples were classified into three groups (Mediterranean–Tarifa, Atlantic and Indo–Pacific) using a heterogeneity test of mtDNA genotype distributions. Combined analysis of the *CaM* and *D-loop* loci indicated that there are at least four breeding units: Mediterranean, northwestern Atlantic, tropical to South Atlantic, and Indo–Pacific.

Key words: swordfish; intron; mtDNA; breeding units.

INTRODUCTION

The swordfish *Xiphias gladius* L., is a highly migratory cosmopolitan fish distributed in a very wide area through tropical to cold waters (Nakamura, 1985). Yet mtDNA assays have indicated that the swordfish population is genetically structured (Kotoulas *et al.*, 1995; Rosel & Block, 1995; Alvarado-Bremer *et al.*, 1996; Chow *et al.*, 1997). These studies show that mtDNA genotype frequency distributions are significantly different among samples derived from the Mediterranean, the Atlantic and Pacific Oceans. However, no heterogeneity of mtDNA genotype frequency has been reported within the Pacific or between the Indian and Pacific Oceans (Grijalva-Chon *et al.*, 1994; Rosel & Block, 1996; Chow *et al.*, 1997). In contrast, mtDNA genotype frequencies are significantly different between North and South Atlantic samples (Alvarado-Bremer *et al.*, 1996; Chow *et al.*, 1997). Thus, mtDNA analysis has revealed genetic heterogeneity between samples, but its haploid nature gives it

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little power for substantiating a sample as a subsample from a panmictic unit and/or for handling population admixture.

Intron-targeted PCR was introduced to find neutral DNA polymorphisms in nuclear genes (Corte-Real *et al.*, 1994; Schlee *et al.*, 1996). The strategy relies on PCR primers designed from highly conserved protein-coding exon sequences flanking more variable non-coding intron regions. Recently, Chow (1998) designed a set of PCR primers to amplify the fourth intron of the calmodulin gene, and demonstrated its universality to the extent that clean amplification of a few fragments was obtained in a wide variety of fish species as well as in man. Chow (1998) proposed that the primer set was amplifying a polymorphic intron within a single copy of the calmodulin gene locus in the swordfish. This was because amplification of a single fragment was observed and restriction fragment length polymorphism (RFLP) of this fragment produced genotypes that were accordance with the Hardy–Weinberg equilibrium in a Mediterranean sample (Chow, 1998). However, there are several gene copies encoding calmodulin in human (Koller *et al.*, 1990) and in medaka *Oryzias latipes* (Temminck & Schlegel) (Matsuo *et al.*, 1992). In order to substantiate the RFLP proposed by Chow (1998), DNA fragments amplified by the universal PCR primers were cloned and sequenced. In order to investigate further global stock structure of the swordfish, larger sample sizes from a wider geographic range were subjected to RFLP analysis using this novel nuclear gene marker together with a previously described mtDNA marker. Here the results are reported of genetic analyses which revealed significant genetic differentiation between swordfish samples, and the distribution of breeding units is discussed.

MATERIALS AND METHODS

Collection information for the swordfish samples is presented in Table I. Individuals were juvenile or adult. Sub-codes with asterisk indicate samples used by Chow *et al.* (1997), from which data on mtDNA D-loop variation were derived. Since two endonucleases (*Alu* I and *Rsa* I) of the four used by these authors appeared to provide better resolution than the others, these two enzymes were applied to newly collected samples in this study. Additional individuals from the Mediterranean Sea (sub-code Medit2) were collected in the same year as those in the previous study; a new combined sample set having a larger sample size was created as the Mediterranean representative. Newly collected samples from the northwest Atlantic (NWA97) and from Brazil (Brazil96) were added, respectively, to previous NWA and Brazil samples. Likewise, the Seychelles sample was combined with two previous Indian samples (Sri Lanka and SJava). Since previous PCR-RFLP assay detected no heterogeneity in the mitochondrial genotype distributions among samples collected in the Pacific (Chow *et al.*, 1997), all Pacific samples were pooled to generate a single sample set (Pacific). Nuclear DNA analysis was performed on two geographically distant samples from the Pacific (Japan and Peru).

DNA was extracted from muscle using a standard phenol/chloroform method and subjected to the polymerase chain reaction (PCR). Primers for amplifying a segment between the 3′ region of exon 4 and 5′ region of exon 5 containing the entire intron 4 of the calmodulin gene (*CaM*) were designed by Chow (1998), and their sequences are 5′-CTGACCATGATGGCCAGAAA-3′ (CALMex4F) and 5′-GTTAGCTTCTCCC CCAGGTT-3′ (CALMex5R). The PCR reaction mixture (a final volume of 8 µl) contained 0·2 U *Taq* DNA polymerase (Perkin-Elmer Cetus, Norfolk, U.S.A.), 0·2 mM of each dNTP, 0·8 µl of manufacturer's supplied 10 × buffer, 2 mM $MgCl_2$, 10 pmol of each primer and 10–50 ng of template DNA. Amplification was carried out with

TABLE I. Descriptions of samples of swordfish *Xiphias gladius* used in this study

Ocean	Sample code	Locality caught	Latitude	Longitude	Sample size	Date	Sub-code
Mediterranean Sea	Medit	Mediterranean	n/a	n/a	34	July 1994	Medit1*
	Medit	Mediterranean	n/a	n/a	47	July 1994	Medit2
North Atlantic	Tarifa	West of Gibraltar Strait	n/a	n/a	39	Aug. 1990–July 1991	Tarifa
	NWA	Northwest	38–40N	59–72W	27	June–Oct. 1990	NWA90*
	NWA	Northwest	20–30N	57–90W	29	Jan.–April 1993	NWA93*
	NWA	Northwest	37–41N	48–67W	19	April–June 1997	NWA97
	TNA	Tropical central north	5–8N	8–21W	30	Jan.–March 1997	TNA
South Atlantic	TSA	Tropical central south	5–11S	2E–8W	56	Jan.–Feb. 1997	TSA
	Brazil	Temperate south	20–30S	35–50W	68	Oct. 1994–Jan. 1995	Brazil94*
	Brazil	Temperate south	20–33S	28–46W	61	Sept. 1996	Brazil96
Indian	Indian	Tropical northeast	n/a	n/a	28	March–April 1992	SriLanka*
	Indian	Tropical southeast	16–17S	118–119E	35	Feb. 1995	SJava*
	Indian	Tropical west	n/a	n/a	21	June 1997	Seycheles
Pacific	Pacific	Northwest	24–35N	144–154E	45	Nov. 1991–Feb. 1992	Japan*
	Pacific	Tropical central north	0–12N	165W	51	Oct. 1994	Hawaii*
	Pacific	Northeast	24–27N	113–115W	35	Jan.–March 1992	Mexico*
	Pacific	Tropical east	1N	79W	21	Oct. 1994	Ecuador*
	Pacific	Southeast	8–18S	84–124W	53	June–July 1994	Peru*
	Pacific	Southwest	30–38S	154–179E	31	June 1994, July–Sep. 1995	SWPO*

*Organizations which supported collection for these samples are shown in Chow *et al.* (1997). Two Pacific samples (Japan and Peru) were used for nDNA analysis as Pacific representative in this study.

an initial denaturation at 95° C for 1 min, followed by 30 cycles of amplification (denaturation at 95° C for 0·5 min, annealing at 60° C for 1 min and extension at 72° C for 2 min) with a final extension at 72° C for 10 min. Under these conditions, a single fragment was amplified in the swordfish, and a very simple restriction profile by *Bst* UI digestion was observed (Chow, 1998). Two individuals from the Mediterranean Sea and one from the Southeast Pacific Ocean were selected for cloning and nucleotide sequencing. RFLP analysis indicated that one (SW19) from the Mediterranean and one (KH5) from the Pacific did not have the *Bst* UI site, and were likely to be *AA* homozygotes. The other individual (SW7) from the Mediterranean had the *Bst* UI site, and was likely to be a *BB* homozygote. Cloning of PCR products was performed using a TA cloning vector, PUCII (3·9 kbp) (Invitrogen, San Diego, U.S.A.) without further purification. Plasmids isolated from *Escherichia coli* by a general method (Sambrook *et al.*, 1989) were digested with *Eco* RI, and those containing target PCR products were screened. The nucleotide sequences of the inserted PCR products were determined by automated sequencer (Applied Biosystems model 373A, U.S.A.) using a *Taq* dye terminator cycle sequencing kit (Perkin-Elmer Cetus, Norfolk, U.S.A.) with M13 reverse and M13 (− 20) forward primers and those used for amplification. Two clones per individual were sequenced. For the RFLP analysis, PCR products of the calmodulin gene intron were digested with *Bst* UI endonuclease (see Chow, 1998) and those of mtDNA D-loop region were digested by *Alu* I and *Rsa* I (see Chow *et al.*, 1997). Digested samples were electrophoresed on a 2·5% agarose gel (Biogel, BIO101 Inc.) in TBE buffer (25 mM Tris, 0·5 mM EDTA, and 25 mM boric acid).

χ^2 analysis was conducted using the Monte Carlo simulation of Roff & Bentzen (1989) with 1000 randomization of the mtDNA genotype data to test heterogeneity between samples. Wright's (1965) fixation indices F_{IS} and F_{ST} were calculated for the *CaM* data to estimate deviations from Hardy–Weinberg proportions in each sample and the degree of genetic differentiation among samples, respectively. These statistical analyses were performed preliminarily to test heterogeneity between samples within locality, and no heterogeneity was observed between samples (data not shown), which justified combining samples within a locality.

RESULTS AND DISCUSSION

VARIATION IN THE CAM LOCUS

Identical nucleotide sequences were observed between the two clones obtained from a Mediterranean individual (SW7) which was supposed to be a *BB* homozygote following *Bst* UI digestion. The nucleotide sequence of clone designated SW7G (Fig. 1), a 504 bp fragment, consisted of two primer sequences (20 bp each, boxed), 3′ partial sequence of exon 4 (58 bp, underlined), entire intron 4 (358 bp) and 5′ partial sequence of exon 5 (48 bp, underlined). The exon region sequences of the swordfish were compared with those of medaka *CaM-A* (Matsuo *et al.*, 1992) and human *CaM-III* (Koller *et al.*, 1990), and showed *c.* 90% homology with both. Deduced amino acid sequences of these three species were identical, and almost all nucleotide substitutions among these three sequences were observed at the third codon. The two clones in SW19 and those in KH5 had the same composition and sequence, but differed from those of SW7 in one nucleotide at 426 bp (identified by an asterisk in Fig. 1). SW19 and KH5, both supposed to be *AA* homozygotes, had thymine at this position, whereas SW7 had cytosine; this nucleotide substitution appears to be responsible for the RFLP revealed by *Bst* UI, as this endonuclease recognizes the CGCG palindrome. A fragment having this recognition site was redefined as the *B* allele and that lacking this site as the *A* allele. The restriction profiles of individual samples

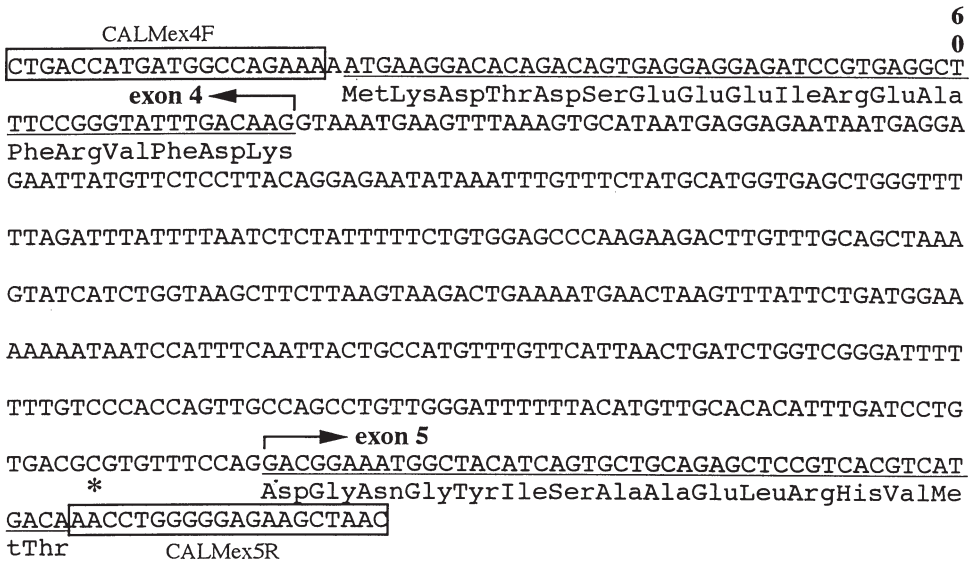

FIG. 1. Nucleotide sequence of a clone (SW7G) amplified universal calmodulin primer. Deduced amino acid sequences are presented beneath the underlined nucleotides. Asterisk (*) indicates variable nucleotide position between alleles *A* and *B*. This fragment is allele *B*, and the variable nucleotide position is substituted by thymine in allele *A*. The nucleotide sequences of *A* and *B* alleles are available in GenBank under the accession number of AF069912 and AF069913, respectively.

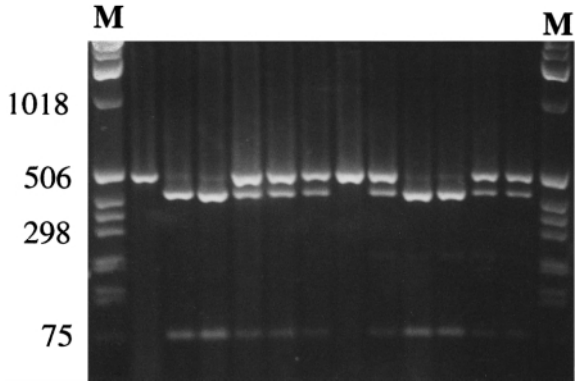

FIG. 2. Restriction profile of *CaM* gene fragment digested by *Bst* UI. M: molecular marker (1 kb DNA ladder, GIBCO BRL). Undigested sample was loaded in the second most left lane. Three genotypes were observed, from which two alleles (*A* and *B*) were inferred. A genotype having only upper single fragment (504 bp) in homozygote of *A* allele, another having two fragments (426 and 78 bp) is homozygote of *B* alleles, and the other having all fragments is heterozygote.

digested by *Bst* UI (Fig. 2), show the three predicted genotypes (*AA*, *AB* and *BB*). However, since amplification of multiple fragments has been reported in other fish species (Chow, 1998), it is still possible that these fragments are from different loci. Using cDNA analysis, Matsuo *et al.* (1992) found four synonymous *CaM* genes (*CaM-A*, *-B*, *-C* and *-D*) in medaka. Homology investigation between exon regions of medaka genes and those of swordfish revealed that medaka *CaM-A* had a higher homology with the swordfish sequence than with

TABLE II. Genotype and allele frequencies at *CaM* locus of eight swordfish samples

Genotype	Pacific*	Indian	Brazil	TSA	TNA	NWA	Tarifa	Medit
AA	95	83	101	34	25	18	7	10
AB	0	1	25	11	5	36	20	30
BB	0	0	2	2	0	19	11	32
n	95	84	128	47	30	73	38	72
A	1	0·994	0·887	0·840	0·917	0·493	0·447	0·347
B	0	0·006	0·113	0·160	0·083	0·507	0·553	0·653
F_{IS}	0	− 0·006	0·030	0·127	− 0·091	0·014	− 0·064	0·081
P	1	0·956	0·753	0·382	0·619	0·708	0·691	0·493

*Japan and Peru samples were chosen for this analysis.

its synonyms (*CaM-B*, *-C* and *-D*). Similarly, the exon sequence of human *CaM-III* reported by Koller *et al.* (1990) had a higher homology with swordfish than with human *CaM-I* reported by Rhyner *et al.* (1994). This is understandable, since the primers used were based on medaka *CaM-A* and human *CaM-III*. Only one nucleotide substitution was observed between the intron regions of the postulated *A* and *B* alleles of the swordfish *CaM*. In contrast, length and nucleotide sequence in the fourth intron regions of the human *CaM-I* and *-III* were considerably different from one another, to the extent that the two intron sequences could not be aligned. These considerations indicate that the primer set used in this study is exclusively amplifying the PCR product from a single gene locus in the swordfish.

The genotype and allele frequencies of the eight samples (Table II) were in accordance with Hardy–Weinberg expectations (F_{IS}= − 0·006 to 0·127, P=0·382 to 1), further supporting the conclusion that these variants really are Mendelian markers. However, considerable heterogeneity in allele frequencies was observed among samples. The frequency of the *A* allele was much lower in the Mediterranean, Tarifa and NWA samples (34·7–49·3%) than in the tropical and South Atlantic (TSA) samples (84·0–91·7%). Furthermore, Indo–Pacific samples were nearly fixed for this allele. The fixation index (F_{ST}) among all eight samples deviated significantly from zero (F_{ST}=0·369, P<0·001) (Table III), indicating that the population consisted of several genetically different breeding units. No significant deviation of F_{ST} from zero was observed among the three samples from the Mediterranean to the North Atlantic (Mediterranean, Tarifa and NWA: F_{ST}=0·023, P>0·5) or among the five samples from the Indo–Pacific to the tropical Atlantic (Pacific, Indian, Brazil, TSA and TNA: F_{ST}=0·071, P>0·1). However, incorporation of any tropical and south Atlantic samples into the Medit, Tarifa and NWA samples resulted in a highly significant deviation of F_{ST} from zero, as well as incorporation of NWA into other Atlantic samples (TNA, TSA and Brazil) (F_{ST}=0·228, P<0·001) or into other Atlantic and Indo–Pacific samples (F_{ST}=0·285, P<0·001). These results indicate that allele frequencies at the *CaM* locus of the NWA, Tarifa, and Mediterranean samples are significantly heterogeneous from other samples.

TABLE III. Genetic differentiation among samples estimated by fixation index (F_{ST}) for *CaM* locus

Sample combination	No. sample	No. individual	F_{ST}	P
Total	8	567	0·369	<0·001
Medit+Tarifa+NWA	3	183	0·023	>0·5
Medit+Tarifa+NWA+TNA	4	213	0·255	<0·001
Medit+Tarifa+NWA+TNA+TSA	5	260	0·269	<0·001
Medit+Tarifa+NWA+TNA+TSA+Brazil	6	388	0·284	<0·001
NWA+TNA+TSA+Brazil	4	278	0·228	<0·001
Pacific+Indian+Brazil+TSA+TNA	5	384	0·071	>0·1
Pacific+Indian+Brazil+TSA+TNA+NWA	6	457	0·285	<0·001

VARIATION IN mtDNA D-LOOP

The genotypes in each restriction digestion observed in the present study were the same as those reported by Chow *et al.* (1997). Frequency distributions of each restriction type and composite genotypes are summarized in Fig. 3. Original data sets of mtDNA genotypes can be seen at our web site (www.enyo.affrc.go.jp/~chow/SWmtDNAtable.html). *Alu* I and *Rsa* I digestions detected four (*A–D*) and eight (*A–H*) genotypes, respectively. In *Alu* I digestion, the frequency of *Alu* I-*C* was lower in the Pacific (0·203) and Indian (0·215) samples than in the Atlantic and Mediterranean samples (0·365–0·642). A similar tendency was observed following *Rsa* I digestion. The frequency of *Rsa* I-*C* was lower in Pacific (0·398) and Indian (0·492) samples than Atlantic samples (0·508–0·698). A much higher frequency of *Rsa* I-*C* was observed in the Tarifa sample (0·949), and all individuals of the Mediterranean sample ($n=71$) were *Rsa* I-*C*. Kotoulas *et al.* (1995), using a mtDNA assay, were the first to describe the genetic uniqueness of Mediterranean swordfish. They suggested that the rate of gene flow between Mediterranean and Atlantic is low enough to prevent their genetic homogenization. The results of Chow *et al.* (1997) and the present study provide further genetic evidence of the unique genetic status of the Mediterranean swordfish stock; no exogenous immigrants have been detected so far in this body of water. Kotoulas *et al.* (1995) also suggested that the sample from Atlantic side of the Strait of Gibraltar (Tarifa) is a mixture of Atlantic and Mediterranean individuals, but dominated by the latter. This is completely consistent with the present results, in which only two of 39 individuals of Tarifa sample were not *Rsa* I-*C*, and it is highly probable that these two might originate from other breeding units. Combining these *D-loop* restriction types yielded 23 genotypes in 688 individuals. Genotype *Alu* I/*Rsa* I-*CC* was rare in the Indo–Pacific (0·06) but common in the Atlantic and Mediterranean (0·20–0·46). Prior to performing heterogeneity tests on the genotype distributions between samples, some samples were pooled. The Mediterranean and Tarifa samples were pooled, since they were almost identical in allele and genotype frequencies of both nDNA and mtDNA. TNA, TSA and Brazil were pooled, as they were considerably different from NWA in allele frequencies at the *CaM* locus and geographically separated from the Indo–Pacific. Indian and Pacific samples

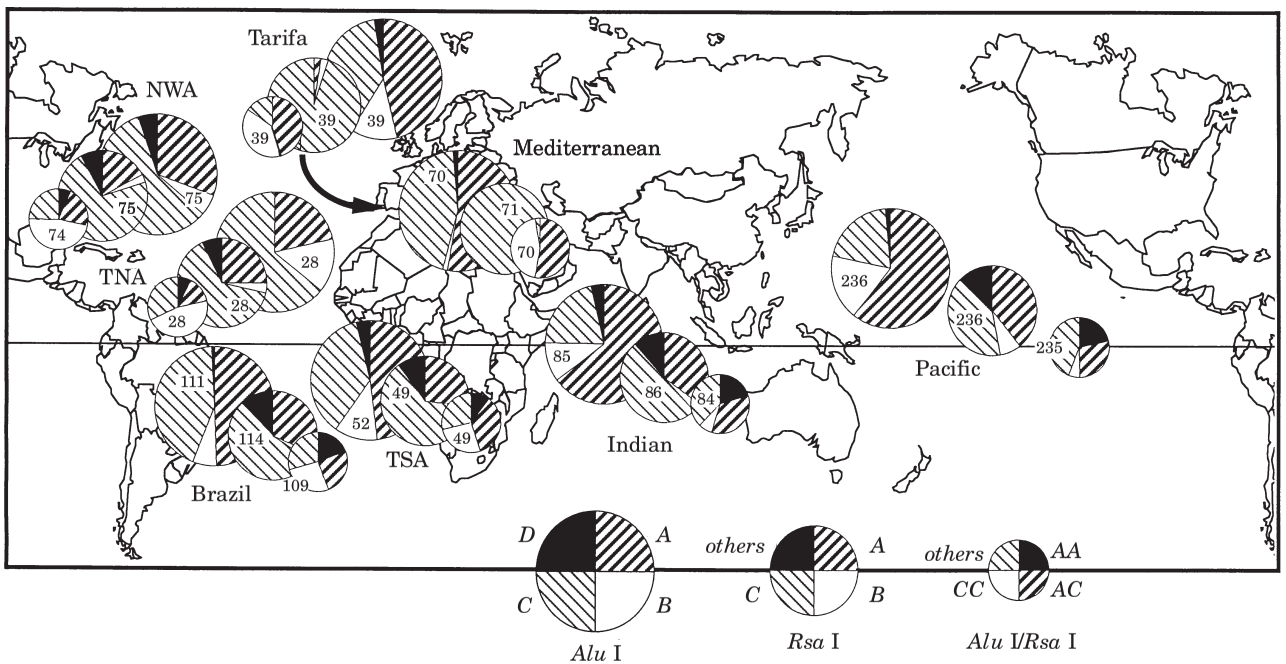

FIG. 3. Pie graph representation of restriction type and genotype frequencies of mtDNA *D-loop* locus. Number in each pie indicates number of individuals examined.

TABLE IV. Results of chi-square analysis for *D-loop* genotype data between samples. χ^2 value (below diagonal) and *P* (above diagonal)

	Indo–Pacific	Brazil–TSA–TNA	NWA	Tarifa–Medit
Indo–Pacific		<0·001	<0·001	<0·001
Brazil–TSA–TNA	75·278		0·137	<0·001
NWA	107·307	21·311		<0·001
Tarifa–Medit	142·396	63·070	43·936	

were pooled, since no genetic heterogeneity between them has been observed so far (Alvarado-Bremer *et al.*, 1996; Chow *et al.*, 1997). Furthermore, the monomorphic feature at *CaM* locus in the Indo–Pacific samples may justify this manipulation. Among the six pair-wise comparisons, all but one (NWA *v.* tropical to South Atlantic) showed heterogeneity (Table IV). Another grouping for the Atlantic samples was attempted by pooling the TNA sample with NWA. But no significant difference was observed between the North (NWA and TNA) and South (TSA and Brazil) Atlantic samples ($P=0·067$). Although the present study analysed larger sample sizes from wider localities in the Atlantic than did the studies of Alvarado-Bremer *et al.* (1996), and Chow *et al.* (1997), present mtDNA data failed to reveal clear differences between North and South Atlantic samples. It is highly probable that the two samples used in previous studies (Alvarado-Bremer *et al.*, 1996; Chow *et al.*, 1997), which did show mtDNA frequency differences, may be two extremes in a clinal distribution of mtDNA genotype frequencies. However, the *CaM* data did reveal sharp difference between NWA and other Atlantic samples. Thus the *CaM* and *D-loop* data are not completely consistent with one another but rather appear to be complementary for delineating swordfish population structure.

ASSORTMENT OF CAM GENOTYPES AND FURTHER IMPLICATION FOR POPULATION STRUCTURE

It is not clear whether the samples used in this study are sub-samples from panmictic units or result from population admixture. While all samples were in Hardy–Weinberg equilibrium, a mixture between genetically differentiated stocks does not always result in significant departures from equilibrium. The likelihood of detecting such a mixture depends on the magnitude of the allele frequency difference between the contributing stocks and the mixing ratio. Given that all Mediterranean swordfish possess the *Rsa* I-*C* type for the *D-loop*, removing *Rsa* I-*C* individuals from a sample may create a new sample set free of Mediterranean individuals. The composite genotype *Alu* I/*Rsa* I-*CC* in *D-loop* locus was very rare in Indo–Pacific samples, so that a sample set only with *Alu* I/*Rsa* I-*CC* individuals may be nearly free of Indo–Pacific individuals. Three data sets were created: *CaM* genotypes assorted with *Rsa* I-*C* individuals, *CaM* genotypes assorted with no *Rsa* I-*C* individuals, and *CaM* genotypes assorted with *Alu* I/*Rsa* I-*CC* individuals (Table V). No marked changes in allele frequency were observed between data sets within local samples, indicating that stock mixture if any is not significant. Since a higher frequency of the *CaM-B*

TABLE V. Genotype frequency at *CaM* locus assorted with mtDNA genotypes

D-loop genotypes	*CaM* genotypes	Indian, Pacific	Brazil	TSA	TNA	NWA	Tarifa, Medit
Rsa I-*C*	*AA*	76	51	22	15	12	16
	AB	1	8	6	3	27	49
	BB	0	2	1	0	13	43
	n	77	61	29	18	52	108
	A	0·994	0·902	0·862	0·917	0·490	0·375
	B	0·006	0·098	0·138	0·083	0·510	0·625
	F_{IS}	− 0·010	0·261	0·130	− 0·090	− 0·040	0·032
	P	0·954	0·042	0·484	0·700	0·779	0·739
No *Rsa* I-*C* (no Mediterranean)	*AA*	90	37	11	8	6	1
	AB	0	16	6	2	9	1
	BB	0	0	1	0	5	0
	n	90	53	18	10	20	2
	A	1	0·849	0·778	0·900	0·525	0·750
	B	0	0·151	0·222	0·100	0·475	0·250
	F_{IS}	0	− 0·180	0·036	− 0·110	0·098	− 0·330
	P	1	0·196	0·880	0·725	0·662	0·637
Alu I/*Rsa* I-*CC* (no Indo–Pacific)	*AA*	12	24	10	10	7	8
	AB	0	4	1	3	12	22
	BB	0	1	1	0	6	16
	n	12	29	12	13	25	46
	A	1	0·897	0·875	0·885	0·520	0·413
	B	0	0·103	0·125	0·115	0·480	0·587
	F_{IS}	0	0·256	0·619	− 0·130	0·038	0·014
	P	1	0·167	0·032	0·638	0·848	0·926

allele was observed in the Mediterranean sample, removing *Rsa* I-*C* individuals from samples must result in a decreasing of the *CaM-B* allele frequency, if Mediterranean swordfish are in these samples. However, a slight decrease in *CaM-B* allele frequency was observed only in the NWA sample, indicating that excursions by Mediterranean swordfish are possible to the western side of the North Atlantic but more limited to the tropical and South Atlantic. Although similar *CaM* allele frequencies between Mediterranean and Northwest Atlantic samples may indicate some ongoing or historical gene flow from the Mediterranean towards the Atlantic, any descendants would not have returned to Mediterranean Sea. If Indo–Pacific individuals were present in the Atlantic–Mediterranean samples, samples comprising only *Alu* I/*Rsa* I-*CC* individuals would have a decreased frequency of the *CaM-A* allele. However, a slight decrease of *CaM-A* allele frequency was observed only in the TNA sample, and the small sample size ($n=13$) may be responsible for this. Thus penetration by Indo–Pacific swordfish into the Atlantic seems negligible.

No genetic differentiation was observed between Indian and Pacific samples. There is a water pathway between the Pacific and Indian oceans (Fieux *et al.*, 1994; Meyers *et al.*, 1995; Gordon & Fine, 1996), along which the Indonesian throughflow may transport the North and South Pacific waters to the Indian Ocean through the complex system of the Australasian Mediterranean Seas.

Thus, swordfish larvae and young juveniles of the western tropical Pacific, an important spawning ground of swordfish (Nishikawa *et al.*, 1985), may be transported into the Indian Ocean, making Indian and Pacific swordfish populations homogeneous. In contrast, a more complex stock structure is apparent in the Atlantic. The present nDNA and mtDNA data prove the existence of a separate stock in the northwestern Atlantic, which concurs with ICCAT's (International Commission for the Conservation of Atlantic Tunas) working hypothesis for separating a northwestern Atlantic stock at 10° N and 35° W (Miyake & Rey, 1989), and with the results of tag-recapture (Farber, 1988). Parasite fauna in the swordfish was found to be considerably different between samples from Northwest Atlantic and Gulf of Guinea (Castro *et al.*, 1998). These indicate that the swordfish is much less migratory than expected. The source of the northwestern Atlantic stock may be the Gulf of Mexico–Caribbean, where a high abundance of swordfish larvae has been observed (Nishikawa *et al.*, 1985; Farber, 1988). Although the degree of eastward extension of this stock is not clear, two individuals (not *Rsa* I-*C*) found in the Tarifa sample may indicate that the peripheral edge of the northwestern stock may reach the Strait of Gibraltar. Sample collection from the central north Atlantic followed by genetic analysis is necessary to delineate mixtures between Mediterranean or tropical–South Atlantic and northwestern stocks and to assess the extent and population dynamics of these stocks. Many swordfishes in the tropical and south Atlantic may belong to a stock separable from the Mediterranean, northwestern Atlantic and Indo–Pacific stocks. Spawning of swordfish appears to be widespread throughout the tropical Atlantic, including off Brazil (Nishikawa *et al.*, 1985; Farber, 1988), and this wide area may be a breeding unit for a tropical-South Atlantic stock.

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