# BOUNDARY BETWEEN NORTH AND SOUTH ATLANTIC STOCKS OF SWORDFISH (Xiphias gladius): AN IMPLICATION FROM NUCLEAR DNA ANALYSIS

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#### SUMMARY

Considerable differences in genotype and allele frequencies at the calmodulin gene locus (CaM) were observed between north and south Atlantic swordfish samples. Frequencies of allele A ranged from 0.464 to 0.517 in the north samples, while those in the south ranged from 0.84 to 0.917. This difference was stable over long period of time (6 to 9 years). The boundary zone between the north and south stocks may be located around 10°N to 20°N.

### RÉSUMÉ

Des différences considérables de génotype et de fréquence des allèles du locus du gène calmodulin (CaM) ont été observées entre les échantillons despadon du nord et du sud de lAtlantique. La fréquence des allèles A allait de 0.464 à 0.517 dans les échantillons du nord, alors que ceux du sud allaient de 0.84 à 0.917. Cette différence était stable sur de longues périodes (6 à 9 ans). Il se peut que la ligne de démarcation entre les stocks nord et sud se situe aux alentours de 101N à 201N.

#### RESUMEN

Se observaron diferencias considerables en el genotipo y las frecuencias alélicas en el locus del gene calmodulina (CaM) entre muestras de pez espada del Atlántico norte y del Atlántico sur. Las frecuencias del alelo A oscilaron entre 0,464 y 0,517 en las muestras del norte, mientras que las del sur oscilaron entre 0,84 y 0,917. Esta diferencia fue estable a lo largo de un amplio periodo de tiempo (de 6 a 9 años). La zona límite entre los stocks del norte y el sur podría estar localizada entre los 10°N y los 20°N.

#### KEYWORDS

Swordfish, Nuclear DNA, Atlantic Stocks, Boundary

Several genetic studies have indicated swordfish population to be structured between ocean basins and also within Atlantic (Kotoulas *et al.* 1995, Rosel and Block 1995, Alvarado-Bremer *et al.* 1996, Chow *et al.* 1997, Chow and Takeyama 2000). Especially, genetic variation found at the calmodulin gene locus (*CaM*) based on a single nucleotide polymorphism (SNP) has revealed considerable heterogeneity in allele and genotype frequencies between Northwest Atlantic-Mediterranean samples and tropical North to South Atlantic samples (Chow and Takeyama 2000), where the frequency of allele *A* in the former samples ranged from 0.347 to 0.493 and that in the later 0.840 to 0.917. Their results indicated that North and South Atlantic stocks of swordfish are genetically isolated and suggested that the boundary between the stocks may be around 10°N. This simple genetic marker may be used as a feasible tool for further delineating the stock structure and mixing between different stocks in the Atlantic. Yet, in order to substantiate the feasibility, temporal variation of the genotype and allele frequencies within and between localities has to be investigated. Here, we report the results of our analysis on the genetic variation at the *CaM* gene locus of supplemental swordfish samples collected in 1998 and 1999 from Northwest and central Atlantic.

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Two supplemental samples were from Northwest Atlantic (NWA98) collected in 1998 at 35° to 43°N and 45°W to 71° W and tropical North Atlantic (TNA99) collected in 1999 at 2°N to 10°N and 21°W to 33°W. Procedures for PCR amplification and restriction analysis for *CaM* gene locus are described in Chow and Takeyama (2000). Fixation indices ( $F_{IS}$  and  $F_{ST}$ )(Wright, 1965) were calculated for *CaM* data for estimating deviations from Hardy-Weinberg proportions and the degree of genetic differentiation among samples. The significant tests for these fixation indices were performed by the formulae  $\chi^2 = NF_{IS}^2$  and  $\chi^2 = 2NF_{ST}^2$ , where *N* is number of individual (Pamilo and Varvio-Aho 1984, Workman and Niswander 1970).

Two alleles (*A* and *B*) were observed in these two supplemental samples, and genotype and allele frequencies together with those of previous study (Chow and Takeyama 2000) are shown in Table 1. Genotype distributions of each sample are in accordance with Hardy-Weinberg expectations ( $F_{IS}$ =-0.149 to 0.250, P>0.25), while those of the entire sample significantly deviated from the expectations ( $F_{IS}$ =0.204, P<0.001). Large excess of homozygotes appears to be responsible for this deviation from Hardy-Weinberg equilibrium, indicating that the entire Atlantic swordfish population consists of genetically different stocks. Allele frequency analysis of various combinations of the eight samples revealed that the significant heterogeneity appeared to be caused by the steep difference of allele frequency between four Northwest Atlantic samples and four tropical to South Atlantic samples ( $F_{ST}$ =0.002, P>0.9) nor among the four tropical to South Atlantic samples ( $F_{ST}$ =0.002, P>0.9) nor among the four tropical to South Atlantic samples ( $F_{ST}$ =0.002, P>0.9) nor among the four tropical to South Atlantic samples ( $F_{ST}$ =0.002, P>0.9) nor among the four tropical to South Atlantic samples ( $F_{ST}$ =0.002, P>0.9) nor among the four tropical to South Atlantic samples ( $F_{ST}$ =0.002, P>0.9) nor among the four tropical to South Atlantic samples ( $F_{ST}$ =0.002, P>0.9) nor among the four tropical to South Atlantic samples ( $F_{ST}$ =0.002, P>0.9) nor among the four tropical to South Atlantic samples ( $F_{ST}$ =0.002, P>0.9) nor among the four tropical to South Atlantic samples ( $F_{ST}$ =0.01, P>0.1), while the difference between pooled samples from the Northwest and South Atlantic was highly significant ( $F_{ST}$ =0.35, P<0.001).

The present results indicate that there are at least two distinct swordfish stocks in the Atlantic; one in the North higher than 20°N and the other in the South lower than 10°N, and the allele frequencies are stable within each stocks throughout somehow long time period (1990 to 1998 in the North and 1994 to 1999 in the South). In order to assess the extent and dynamics of these stocks around the postulated boundary zone, we recommend intensive Atlantic-wide sample collection around 10°N and 20°N followed by the genetic analysis using the *CaM* gene marker.

## LITERATURE CITED

- ALVARADO-BREMER, J. R., J. Mejuto, T. W. Greig and B. Ely. 1996. Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. J. Mar. Biol. Ecol. 197: 295-310.
- CHOW, S., H. Okamoto, Y. Uozumi, Y. Takeuchi and H. Takeyama. 1997. Genetic stock structure of the swordfish (*Xiphias gladius*) inferred by PCR-RFLP analysis of the mitochondrial DNA control region. Mar. Biol. 127: 359-367.
- CHOW, S. and H. Takeyama 2000. Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish (*Xiphias gladius*). J. Fish Biol. 56:1087-1098.
- KOTOULAS, G., A. Magoulas, N. Tsimenides and E. Zouros. 1995. Marked mitochondrial DNA differences between Mediterranean and Atlantic populations of the swordfish, *Xiphias gladius*. Mol. Ecol. 4: 473-481.
- PAMILO, P. and S. Varvio-Aho. 1984. Testing genotype frequencies and heterozygotes. Mar. Biol. 79: 99-100.
- ROSEL, P. E. and B. A. Block. 1995. Mitochondrial control region variability and global population structure in the swordfish, *Xiphias gladius*. Mar. Biol. 125: 11-22.
- WORKMAN, P. L. and J. D. Niswander. 1970. Population genetic studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am. J. Hum. Gen. 22: 24-49.
- WRIGHT, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395-420.

	NWA98	NWA97*	NWA93*	NWA90*	TNA99	TNA97*	TSA97*	Brazil94-96*
	35-43N	37-41N	20-30N	38-40N	2-10N	5-8N	5-11S	20-33S
Genotype	45-71W	48-67W	57-90W	59-72W	21-33W	8-21W	2E-8W	28-50W
AA	15	5	8	5	68	25	34	101
AB	27	6	14	16	15	5	11	25
BB	14	5	7	7	1	0	2	2
n	56	16	29	28	84	30	47	128
Α	0.509	0.5	0.517	0.464	0.899	0.917	0.840	0.887
В	0.491	0.5	0.483	0.536	0.101	0.083	0.160	0.113
$F_{IS}$	0.035	0.25	0.033	-0.149	0.018	-0.091	0.127	0.028

Table 1. Genotype and allele frequencies at CaM locus of eight Atlantic swordfish samples.

Data with asterisk are derived from Chow and Takeyama (2000).