

## STOCK DIFFERENTIATION OF ATLANTIC SWORDFISH USING MITOCHONDRIAL DNA ANALYSES

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## SUMMARY

This paper reports the preliminary results of the study of mtDNA variation of swordfish (*Xiphias gladius*) aimed to recognize population stocks in the North Atlantic. Studies on the analysis of fragment length polymorphisms in mitochondrial DNA (mtDNA) have proved useful in the recognition of population stocks of many animals. Each stock exhibits a few characteristic variants of mtDNA genomes separated by very small numbers of mutational steps (values of  $p < 1$  percent). Analyses on six swordfish specimens from Georges Bank revealed comparatively large values of sequence divergence ( $p = > 1.6$  percent), suggesting that populations stocks are well structured elsewhere. For comparative purposes, the genetic variation and the phylogenetic relationship of five members of Istiophoridae were assessed. Currently, the genetic variation of samples from other North Atlantic localities is being studied. International cooperation in the collection of samples is stressed.

## RESUME

Le présent document fait état des résultats préliminaires de la variation de l'ADN mitochondrial de l'espadaon (*Xiphias gladius*) visant à identifier les stocks de la populations nord-atlantique. Les études sur l'analyse du polymorphisme de la longueur du fragment de l'ADN mitochondrial se sont avérées utiles pour reconnaître les stocks de nombreux animaux; chaque stock montre quelques variantes caractéristiques des génomes de l'ADN mitochondrial, séparées par un très petit nombre de stades mutatifs (valeur de  $p = 1$  %). L'analyse de six échantillons d'espadaon en provenance du Georges Bank a révélé des valeurs comparativement importantes de divergence séquentielle ( $p = 1.6$  %), ce qui suggère qu'ailleurs les stocks sont bien structurés. La variation génétique et la relation phylogénétique de cinq spécimens d'Istiophoridés ont été évaluées à des fins de comparaison. La variation génétique des échantillons d'autres secteurs de l'Atlantique nord est actuellement sous étude. Nous insistons sur la collaboration à l'échelle internationale pour le recueil d'échantillons.

## RESUMEN

Este documento presenta los resultados provisionales del estudio de la variación del mtDNA del pez espada (*Xiphias gladius*) que tiene por objeto la observación de los stocks en el Atlántico norte. Los estudios sobre el análisis de polimorfismos de talla fragmentada en el DNA (mtDNA) mitocondrial, han resultado útiles para reconocer la población de los stocks de muchos animales. Cada stock presenta algunas variantes características de genomas de mtDNA, separados por muy escasos números de pasos mutacionales (valores de  $p < 1\%$ ). El análisis de muestras de seis peces espada procedentes de Georges Bank, reveló valores de divergencia secuencial relativamente altos ( $p = > 1.6\%$ ), lo que sugiere que los stocks están bien estructurados en otras zonas. Se evaluó la variación genética y la relación filogenética de cinco istiofóridos para establecer una comparación. Actualmente se está estudiando la variación genética de muestras procedentes de otras zonas del Atlántico norte. Se destaca la importancia de una colaboración internacional en la obtención de muestras.

## Introduction

Studies on the analysis of fragment length polymorphism in mitochondrial DNA (mtDNA) have provided valuable information in the recognition of population stocks of many animals (see for example: Avise et al., 1987, Moritz et al., 1987, Van Wagner and Baker 1986, 1990). Since mtDNA is maternally inherited, each cell in every offspring inherits the characteristic mtDNA of the mother, and in turn the female offspring will transfer theirs intact to their offspring. Mutations may occasionally occur in mtDNA of an egg cell (at up to ten times more frequently than in the nuclear genome). If that egg cell is fertilized and develops into a female, all her offspring will also carry that mutation. Therefore the descendant stock of the mutant female may be recognized by analyzing the patterns of variation of the mtDNA of its members. The establishment of new variants is constrained by stochastic mechanisms, so local populations will possess only of a few characteristic variants of mtDNA haplotypes (Avise et al. 1987). If rates of male and female dispersal are equal, gene flow for the mtDNA is only one-fourth that for the nuclear genome (Birky et al. 1983), and thus mtDNA is an extremely sensitive indicator for population subdivision (Wilson et al., 1985).

Except for the unpublished study by Johnson (1987) on protein electrophoresis, there is no information about genetic variation and population genetics of the swordfish (*Xiphias gladius*). On the basis of morphometric information, and the presence of specific parasites, several authors have suggested that swordfish subpopulations may exist (see for example: Silas and Ummerkutty 1967, Radtke and Hurley 1983). Tagging programs, which have less than a 10% recovery, have shed little light on the population structure of the species in the North Atlantic Ocean (see for example Beckett 1974), or elsewhere (Palko et al. 1981). However, tagging programs have provided some valuable information about seasonal movements; females on the west Atlantic may return to the same temperate feeding grounds each year, while males stay farther south, near, or within the warmer waters of the breeding grounds (Beckett 1970). A similar sexual differential distribution is observed in other waters (see Palko et al. 1981, and references

therein). Gene exchange between potentially breeding populations is then presumably confined to tropical waters. Even if only females observe philopatry, studies of mtDNA variation may provide valuable information for the recognition of population stocks. Genetic distances between and within other billfishes, including marlins and sailfishes, were calculated and compared with values of sequence divergence between swordfish specimens.

## Materials and Methods

Mitochondrial DNA was isolated from samples of frozen heart and liver dissected by the author, from swordfish (n=6) captured by the crew of the commercial fishing vessel 'Endurance' during 1-3 October 1989 on Georges Bank, Nova Scotia. Isophorids were sampled during the summer of 1989 on several sport fishing operations on both the Atlantic and Pacific coasts of Mexico. The species and locality of capture are as follows: *Tetrapterus audax* (n= 3) in Cabo San Lucas; *Istiophorus platypterus* (n= 6) and *Makaira mazara* (n=1) in Acapulco; *I. albicans* (n= 6) and *T. albidens* (n= 5) in Cancun. Tissue was dissected within 8 hours of death, and immediately stored in liquid nitrogen and later transferred to a -70°C freezer at the Royal Ontario Museum until they were assayed.

Mitochondrial DNA was isolated from solid tissue using a protocol adapted from Brown (1980) and Lansman et al. (1981) and described in Van Wagner and Baker (1990). Tissue (2g) was minced in petri dishes, ground with several strokes in a Dounce homogenizer, and centrifuged to pellet the nuclei and then the mitochondria. After lysing the mitochondria, the mtDNA was purified in two CsCl density gradients, each run for 10 h at 436 000 x g in a Beckman TI-100 tabletop ultracentrifuge at 4°C. To identify clones, I digested the mtDNA with the following 9 restriction enzymes: *Hind* III, *Nci* I, *Ava* II, *Pvu* II, *Stu* I, *Cla* I, *Sau* 96I, *Mbo* I and *Hinf* I. The fragments were end-labelled with P<sup>32</sup> and separated in 1.2% agarose or 4% polyacrylamide gels, and visualized with autoradiography. For size standards, I used a 1-kb ladder (Bethesda Research

Laboratories). Fragment homologies (F) and percent nucleotide sequence divergences ( $p$ ) were calculated using the formulae of Nei and Li (1979) with the program BIGDNA written in PASCAL by Dr. Alejandro Lynch. These values were clustered with UPGMA cluster analysis to reveal the genetic identity and relationships among populations of swordfish.

### Results

Estimates of the size of the mitochondrial genome of billfish varied from 14 600 to 16 400 bp long. The restriction enzymes generated approximately 62 fragments of swordfish mtDNA, corresponding to 295 recognized bp, or about 2% of the mitochondrial genome. Figure 1 gives an example of the fragments produced by digesting the mtDNA with Sau96 I.

All enzymes produced polymorphic patterns (table I). Five enzymes revealed polymorphisms within species. Two enzymes resolved at species level, two at genus level, and one at family level (table I). For example, Hinf I only separated Istiophoridae from Xiphiidae, while Sau96 I revealed the presence of different haplotypes within species. The estimate of nucleotide divergence for each species is presented off the diagonal on table II. There is a very high nucleotide divergence between Xiphias specimens (1.7%). The level of divergence is higher than between two species of Tetrapterus (1.4%). Values of sequence divergence as high as 58% separated members of Istiophoridae from Xiphias.

Relationships of swordfish, sailfishes and striped and white marlins based on levels of their mtDNA percent sequence divergence are depicted on figure 2. The branches represent the UPGMA cluster analysis of 100 $p$  for 5 taxa (matrix correlation = 0.999). Since the sequence divergence value that separate swordfish from the Istiophoriids is higher than 15%, it is not a reliable estimate of phylogenetic relationships (Avice et al.

1987); it merely reflects the long separate evolutionary histories of these two groups. The relationship of Makaira with other istiophorids, based on the restriction analysis of 6 enzymes, are depicted on figure 3. The estimate of sequence divergence of Makaira from the other Istiophorids is as high as 9%; this estimate may vary by assessing more restriction sites. The clusters depicted in figures 1 and 2 give additional refinement to the phylogenetic relationships established with morphometric and meristic data by Collette et al. (1984) and by Johnson (1986).

### Discussion

Most restriction enzymes resolved well at the specific level, however, four-base-cutter enzymes, such as Mbo I and Hinf I, yielded the highest resolution for distinguishing intraspecific haplotypes. The values of percent nucleotide sequence divergence ( $p$ ), revealed the genetic identity and relationships among species and potentially between populations. Since sequence divergence between Xiphiidae and Istiophoridae is larger than 15%, the mtDNA estimates of divergence are not accurate. However, the results are useful to establish phylogenetic relationships within families and among the populations of each species.

The estimates of sequence divergence for Atlantic and Pacific sailfish is about 1.5%; the divergence of striped and white marlin is even lower at 0.7%; while the sequence divergence between Tetrapterus and Istiophorus, is roughly 6%. Although the values of sequence divergence for Makaira mazara are tentative, they suggest that Tetrapterus and Istiophorus share a common ancestor from which the ancestor of Makaira diverged. The values calculated within genus could be correlated with the formation of the Isthmus of Panama to calibrate the rate of evolution of mtDNA in billfishes.

The estimate of sequence divergence of the swordfish sample from Georges Banks

was as nearly 1.7%. This estimate is higher than the divergence of *Tetrapterus albidens* from *T. audax*, and shows a discontinuous genetic divergence pattern (i.e., many mutational events separate the mtDNA types). Increasing the swordfish sample size will most likely yield even a higher level of genetic divergence. There are no good examples of phylogenetic discontinuities lacking spatial separation (Category II in Avise et al. 1987); those observed, have turned out to be cases of mistaken identity of hybrid populations or sibling species. Intraspecific mtDNA discontinuities are explained primarily as arising from long term geographic isolation; alternatively, 'breaks' could result from the extinction of intermediate maternal lineages in a species widely distributed but with low values of gene flow and dispersal (Avise et al. 1987). Close genetic similarities have been reported for active swimmers with great dispersal capabilities (Graves et al. 1984). Since swordfish are also capable swimmers, then the high level of genetic divergence reported here for a single locality suggests, that the sample consists of individuals belonging to separate breeding stocks. If maternal phylopatry is actually observed, then, sampling females (preferably with ripe ovaries) or larvae on breeding areas, should produce results characterized by a few mtDNA variants and low levels of genetic diversity. Working in cooperation with the Southeast Fisheries Centre USMFS and the University of South Carolina, I am currently analyzing samples from other areas of the Eastern North Atlantic. It is critical to obtain samples in good condition from the Gulf of Mexico and Caribbean, as well as from other areas of the Atlantic Ocean and the Mediterranean. I would like to request all the attendants to this Meeting their cooperation in securing samples for this project.

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**Table I.** Mitochondrial DNA (mtDNA) clones detected with restriction analysis. The numbers 1-9 refer to the following battery of restriction enzymes: 1. *Pvu* II; 2. *Hind* III; 3. *Bst* II; 4. *Dra* I; 5. *Ava* I; 6. *Nci* I; 7. *Mbo* I; 8. *Sau*96 I; 9. *Hinf* I; n indicates the number of fish possessing a particular mtDNA haplotype.

| Species               | Genotypes |   |   |   |   |   |   |   |   |   |
|-----------------------|-----------|---|---|---|---|---|---|---|---|---|
|                       | n         | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| <i>X. gladius</i>     | 1         | A | A | A | A | A | A | A | A | A |
|                       | 1         | A | A | A | A | A | A | A | A | B |
|                       | 1         | A | A | A | A | A | B | B | C | C |
|                       | 1         | B | A | A | A | A | A | C | B | D |
| <i>I. albicans</i>    | 2         | A | A | A | A | A | A | A | D | E |
|                       | 6         | C | B | B | B | B | F | D | E | F |
| <i>I. platypterus</i> | 3         | C | B | C | B | C | C | E | E | G |
|                       | 1         | C | B | B | B | C | D | E | E | G |
|                       | 1         | C | B | C | B | C | C | E | E | H |
| <i>T. albidens</i>    | 1         | C | B | C | B | C | C | E | E | G |
|                       | 2         | E | B | D | C | E | E | G | F | J |
|                       | 1         | E | B | D | C | E | E | G | F | K |
|                       | 1         | E | B | D | C | E | E | G | F | J |
| <i>T. audax</i>       | 1         | E | B | D | C | E | E | F | F | J |
|                       | 2         | E | B | D | C | D | G | F | H | I |
|                       | 1         | D | B | D | C | F | G | F | G | I |

Table II. Sequence divergence  $p$  between billfish species. Numbers 1-6 refer to swordfish specimens 10, 11, 15, 19, 21, all captured on Georges Bank. Numbers 7-10 are *I. albicans*, *I. platypterus*, *T. albidens*, and *T. audax*, respectively. The high values of sequence divergence between swordfish and other billfish are not necessarily accurate (see text). Note that the values of sequence divergence between swordfish specimens is larger than the divergence within the genera *Istiophorus* or *Tetrapterus*. This values (x 100) are plotted in figure 2).

|    | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    |
|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1  | 0.000 |       |       |       |       |       |       |       |       |       |
| 2  | 0.001 | 0.000 |       |       |       |       |       |       |       |       |
| 3  | 0.011 | 0.011 | 0.000 |       |       |       |       |       |       |       |
| 4  | 0.009 | 0.010 | 0.016 | 0.000 |       |       |       |       |       |       |
| 5  | 0.002 | 0.003 | 0.013 | 0.012 | 0.000 |       |       |       |       |       |
| 6  | 0.002 | 0.003 | 0.013 | 0.012 | 0.000 | 0.000 |       |       |       |       |
| 7  | 0.506 | 0.504 | 0.503 | 0.516 | 0.502 | 0.502 | 0.000 |       |       |       |
| 8  | 0.504 | 0.499 | 0.508 | 0.515 | 0.501 | 0.501 | 0.015 | 0.000 |       |       |
| 9  | 0.483 | 0.482 | 0.495 | 0.515 | 0.474 | 0.474 | 0.071 | 0.063 | 0.000 |       |
| 10 | 0.502 | 0.501 | 0.519 | 0.542 | 0.491 | 0.491 | 0.070 | 0.062 | 0.007 | 0.000 |

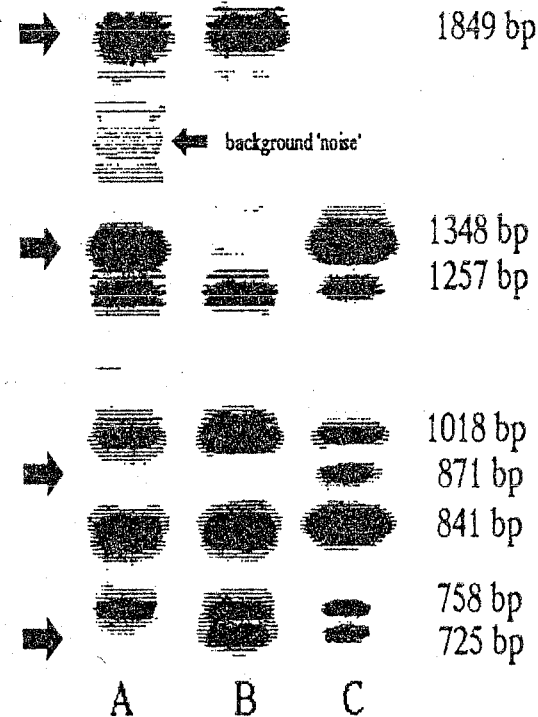
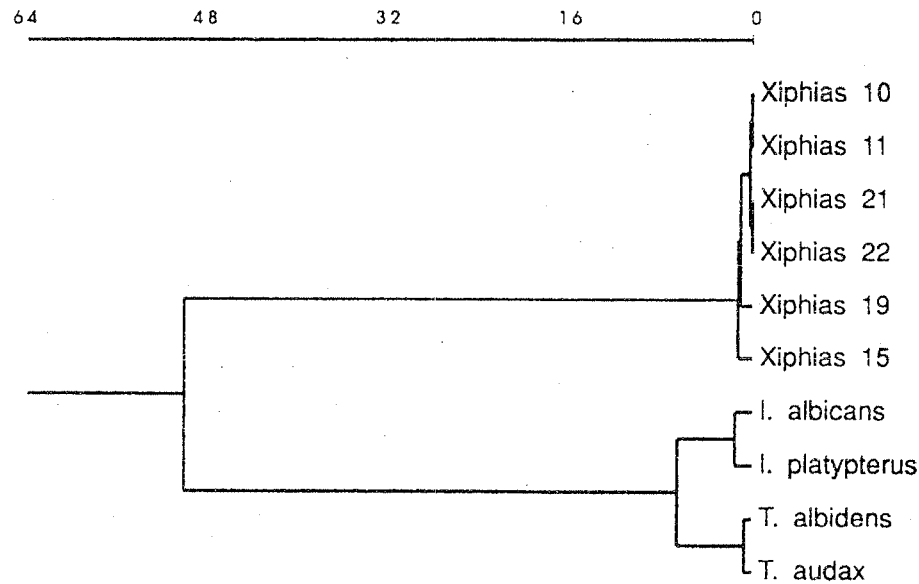


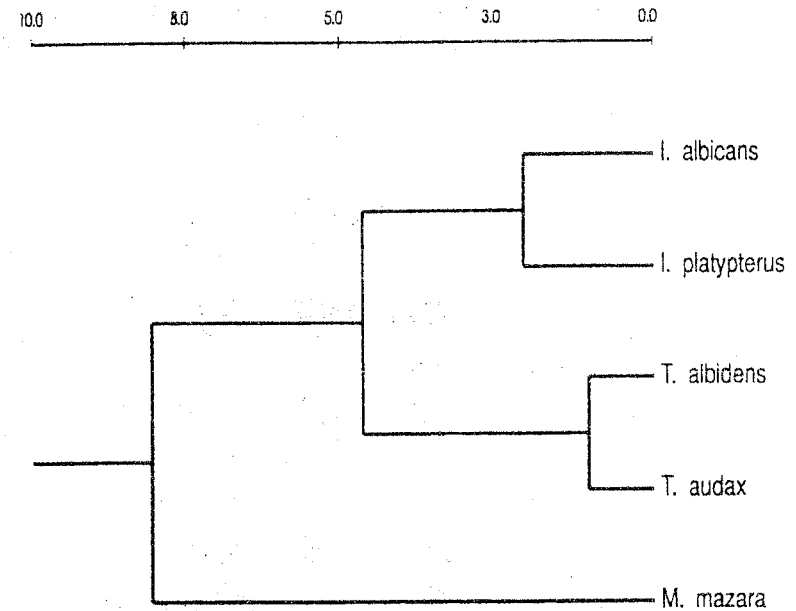
FIGURE 1. Segment of an agarose gel autoradiograph showing three mtDNA haplotypes of swordfish (A, B, and C). The mtDNA molecules were cut with the enzyme *Sau* 96 I and the fragments were then radioactively labelled. *Sau* 96 I cleaves the DNA molecule at sites where the sequence is 5'-GGNC C-3. Each band represents the radioactive signal of a fragment consisting of a certain number of base pairs (bp). The large arrows indicate places where differences among individual fish (A, B, or C) were detected, as demonstrated by the presence or absence of a fragment of a particular size. The three fish were collected on Georges Banks, N. S.

## Percent Nucleotide Divergence



**Figure 2.** Relatedness of the mtDNA of five billfish species. This dendrogram results from the UPGMA cluster analysis of the percentage divergence in table II. The large value of sequence divergence that separates swordfish from Istiophorids may not be accurate (see text). However, results are accurate within the branches of each family. Note that the sequence divergence between swordfish specimens is comparable to that between the two species of *Istiophorus*, or between the two species of *Tetrapterus*.

## Percent sequence divergence



**Figure 3.** Relatedness of the mtDNA of 5 members of the Istiophoridae. This dendrogram is the result of UPGMA cluster analysis of percentage divergence based on only 6 enzymes; because of the small number of enzymes the values of sequence divergence may not be accurate.