Population structure of swordfish in the Pacific Ocean: A review of genetic studies based on the analyses of nuclear and mitochondrial data

by

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Abstract

A review of all published genetic studies of swordfish (*Xiphias gladius)* populations in the Pacific Ocean is presented. In general, the levels of population structuring in the Pacific Ocean are extremely low, compared to other basins. Two studies report significant heterogeneity in the Pacific, while four others found no significant differences. The contrasting views and hypotheses of population structure derived from different kinds of data are given. It is concluded that additional analyses with larger samples sizes and additional genetic markers are needed to resolve the population structure of swordfish in the Pacific Ocean.

Introduction

The stock structure of swordfish in the Pacific is not well known (Hinton 2003, Hinton and Bayliff 2002), but various studies indicate the presence of multiple stocks in the Pacific and possibly in the eastern Pacific Ocean (EPO). Genetic studies of swordfish populations have been conducted with a variety of methods and loci. These include nuclear genomic data, in the form of allozymes, exon-primed amplified intron loci (e.g., *Calmodulin intron 4*). Alternatively, variability within the mitochondrial DNA (mtDNA) genome has been surveyed using restriction fragment length polymorphisms (RFLP) of the entire molecule, or of PCR amplified segments (i.e., PCR-RFLP) and direct nucleotide sequencing. Technique selection, and the assumptions necessary to interpret a particular type of data, falls out of the scope of this review.

Globally, analyses of mitochondrial DNA data have shown inter-oceanic population differentiation in swordfish populations (Alvarado Bremer et al. 1995; 1996; Kotoulas et al. 1995; Rosel and Block 1996; Chow et al. 1997; Greig 2000; Chow et al. 2000). Population studies within the Pacific Ocean have employed a variety of techniques to survey variation contained in the mitochondrial genome, including an RFLP analysis of the entire mtDNA molecule (Grijalva-Chon et al. 1994), PCR-RFLP of the D-loop fragment (Chow et al. 1997l Chow and Takeyama 2000), and direct sequencing of the D-Loop region (Rosel and Block 1996). Specifically, all these studies failed to reveal differences among populations within the EPO. Recently, Reeb et al. (2000), analyzed D-loop sequence data from samples collected on a larger geographical scale across the Pacific Ocean, and described significant differences between samples from the NW Pacific (Japan) and Australia (pooled NW and NE Australian samples). Concordant with previous studies, no differences among samples from the EPO were found.

Studies of population structure in the Pacific Ocean have also surveyed nuclear data. Specifically, the allozyme study of Grijalva-Chon et al.(1996) found significant differences in allele frequency at three loci (*PROT-2*, PROT-3*, and ODH**) between sample from Hawaii and Baja California. By contrast, Chow and Takeyama (2000) analyzing *Calmodulin* (*CaM*) PCR-RFLP data found no differences among samples from the Indian (Sri Lanka, Java and Seychelles) and the Pacific (Japan and Peru) Oceans. It should be noted that *CaM* data demonstrated significant levels of differentiation at a global scale, confirming the differences between the Atlantic, Mediterranean and Indo-

Pacific, and between the North Atlantic and South Atlantic revealed by mtDNA D-loop data. Table 1 summarizes the results from genetic studies conducted so far within the Pacific Ocean. Additional data presented by the authors of this study at the Billfish Symposium, Cairns, Australia, are also included (Alvarado Bremer et al. 2001). As can be seen in Table 1, there are certain conflicting results regarding the level of genetic heterogeneity observed within the Pacific, in marked contrast with the level of structuring observed in the Atlantic Ocean and Mediterranean Sea. To certain extent, some of the differences can be attributed to the resolving power intrinsic to a particular type of data, or to the approach employed to characterize variation (e.g. sequence data versus size polymorphisms). However, demographic factors need also to be considered since they can determine the levels of genetic variability found in nuclear and cytoplasmic DNA. For instance, the conflicting results of mtDNA data and nuclear data regarding the heterogeneity of Hawaii and the eastern north Pacific (see Grijalva-Chon et al. 1994, 1996) may reflect the differences in the mode of inheritance and demographic factors that affect these genomes. Theoretically, a higher resolution of nuclear data over mitochondrial data can be expected when sex ratio (females: males) in the order of 7:1 is observed (Birky et al. 1989). However, although the sex ratios in the Hawaiian and Mexican samples analyzed by Grijalva-Chon et al.(1994; 1996) were skewed towards females, they considered that the magnitude of these differences was not sufficiently large to account for the observed disparity in their results.

One alternative explanation to account for the apparent lack of resolution of the RFLP mtDNA data of Grijalva-Chon et al. (1994), is that these authors could not score small restriction fragments which may have been informative. As an alternative to RFLP of the entire mtDNA molecule , direct sequencing of fast evolving regions has become the method of choice in population studies. Specifically, studies using D-loop data in the Atlantic Ocean indicate separate northern and southern swordfish populations. Based on this success, Reeb et al. (2000) sequenced 281 swordfish collected throughout the Pacific using larger samples and a longer D-loop fragment than the one previously employed by Rosel and Block (1995). Hierarchical analysis of variance (AMOVA; Excoffier et al. 1992) supported significant genetic structuring in the Pacific, particularly arguing in favor of isolation by distance to explain the connectivity between regions. It should be noted that the differences between Hawaii and Mexico reported by Grijalva-Chon (1996) with allozymes were not corroborated by Reeb et al. (2000). Instead, their results are in agreement with Grijalva-Chon (1994) regarding the lack of genetic differentiation of mtDNA. However, it should be underlined that a significant portion of the variance explained in Reeb et al (2000) corresponds to their handling of data in the Australian sample, particularly to the steps which corresponds to pooling two geographically disjunct samples, in this case from two oceans, into a single sample (see Table 1).

Attempting to resolve these conflicting views, Alvarado Bremer et al. (2001) conducted a population structure study of swordfish within the Pacific that included both mitochondrial DNA D-loop sequence data and nuclear DNA data from samples collected in the Central Pacific (Hawaii) and the EPO (Mexico, Ecuador, Peru), and SW Pacific (Australia) (see Table 1). These authors surveyed variation in three nuclear genes, tubulin, *aldolase* b and *lactate dehydrogenase* A (*Ldh*A) *intron* 6 (Greig 2000 and references therein). Among the nuclear loci, only *Ldh*A was polymorphic, and

considering these results together with D-loop sequence data, they concluded the following:

Mitochondrial data: a small (3%) but significant amount of variance differentiates Pacific swordfish populations. Thus, AMOVA rejects the null hypothesis of panmixia for mtDNA within the Pacific, with the Australian sample responsible for the highest level of differentiation. Furthermore, analyses of D-loop data revealed no differences among the samples collected in Ecuador, Peru, Hawaii and Mexico. Thus in general, the mtDNA results of Alvarado et al (2001) corroborate the findings of Reeb et al. (2000). It is important to clarify that both Alvarado Bremer et al. (2001) and Reeb et al (2000) independently characterized the same Australian sample (see Table 1, Note 2).Thus, a portion of the reported heterogeneity in genetic variance within the Pacific Ocean may be due to sampling error.

Nuclear data. AMOVA of *Ldh*A failed to reject the null hypothesis of panmixia for the Pacific samples. However, borderline values of significance were obtained due to incipient levels of differentiation. Specifically, one of the alleles of the *Ldh*A gene (allele 5), was present in Peru, Ecuador and Mexico (pooled n=178) at a frequency ranging between 4-8%, but was not detected in the Hawaiian samples (n=202). To verify whether any of these findings are due to sampling error, it is necessary to analyze the allele frequency of *Ldh*A using larger sample sizes with strict attention to spatial and temporal distribution of these samples in order to maximize information return from additional analyses. If the reported heterogeneity is not due to sampling error, then the genetic data from the *Ldh*A gene may serve to answer several questions:

1) Resolution of north, south and Hawaii stocks: The fishery data analysis indicates two stocks in the eastern Pacific, a north and south with a boundary at about 5°S (Hinton 2003). This boundary is right at the Ecuador – Peru boundary, but well north of Chile. The boundary of a single north Pacific stock has been proposed by Kleiber to be 10 N in analyses of stock status based on fisheries data. If evidence exists to support separate stocks, including a west – central Pacific stock that moves into the EPO at various times of the year, then these factors should be addressed in stock assessments and management recommendations of the IATTC.

2) Identification of stock boundaries using genetic data: if there is a genetically distinct stock in the southern EPO, could it be possible to identify the boundary using genetic data? Ideally, samples analyzed should include some taken during el Niño years (1997-98) and samples after this peak (1999), giving the opportunity to contrast in a hierarchical analysis the potential influence of such oceanographic changes on the distribution of swordfish populations. Future work thus should include samples from different years to test for temporal stability within region.

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Table 1. Genetic studies of swordfish population structure within the Pacific Ocean[1]

Reference:	**Data type**	**Approach/Locus**	**Regions compared**	**Interpretation**	**Comments**
Grijalva-Chon et al. 1994	mtDNA	RFLP entire molecule	Central Pacific (Hawaii) and EPO (Mexico), Western (Japan and China Sea)	No differences	
Grijalva-Chon et al. 1996	Allozymes	26 allozymes/4 polymorphic	Central Pacific (Hawaii) and EPO (Mexico)	Significant differences	Freq. Differences for ODH*, PROT-2* and PROT-3*
Rosel and Block 1996	mtDNA	Sequence/D-loop	EPO (Mexico, Chile), Central Pacific (Hawaii), NW Pacific (Japan, Taiwan)	No differences	Significant differences against Atlantic and Med. samples
Reeb et al. 2000	mtDNA	Sequence/D-loop	NW Pacific (Japan), C. Pacific (Hawaii), EPO (Cal./Mexico, Ecuador), S.W. Pacific (Australia)		See note 2
Chow et al. 1997	mtDNA	PCR-RFLP/D-loop	NW Pacific (Japan), C. Pac.(Hawaii), EPO (Mexico, Ecuador and Peru), SWPO (N. Zealand.	No differences	Significant differences against Atlantic and Med. samples
Chow and Takeyama (2000)	mtDNA nDNA	PCR-RFLP/D-loop and *Calmodulin* intron 4 (*CaM*)	NW Pacific (Japan) and EPO (Peru)	No differences	

Alvarado Bremer et al. 2001	mtDNA nDNA	Sequencing/D-loop Sequencing/*Ldh*A	Hawaii, Mexico, Peru, Ecuador, Australia	D-loop data/Significant LdhA incipient differentiation between EPO and 3 Hawaiian samples	D-loop difference vs Australian sample (note 2). LdhA: Larger samples required

[1]Only studies with at least two Pacific Ocean samples were included in this table.

[2]The Australian sample consist of two geographically disjunct samples (Indian and Pacific Ocean-sides) pooled together.