GENETIC POPULATION STRUCTURE OF ATLANTIC SWORDFISH: CURRENT STATUS AND FUTURE DIRECTIONS


SUMMARY

The genetic population structure of Atlantic swordfish has been studied with mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) data using a variety of laboratory methodologies and analytical approaches. In here, we compare preliminary data for the nuclear genes ldhA and Calmodulin (Cam) from the South Atlantic against published data from other regions. Results from Cam, but not ldhA, confirm the partitioning of NW Atlantic and South Atlantic swordfish populations. The distribution Cam alleles and CR-I haplotypes is homogeneous throughout the entire South Atlantic (South of 5°N) and comparisons with the Indian Ocean confirm the genetic differentiation of these two basins. However, the boundary that separates the North Atlantic from the South Atlantic cannot be delineated with the present data, and additional temporal-spatial sampling is necessary to resolve this issue. It is important to note that the North Atlantic has been characterized primarily using NW Atlantic and Caribbean samples, with very limited sampling coverage of the NE Atlantic. However, because mixing with the Mediterranean swordfish may occur in the NE Atlantic, it is important to delineate the area of contact between these two regions prior to comparing NE and NW Atlantic samples. Using CR-I data, we show that the mixing zone of NE Atlantic and the Mediterranean is restricted to a small zone west of Gibraltar that extends west to 10°W. Future studies of population structure in the North Atlantic should consider this evidence to avoid Type I errors. This paper also underlines other areas of research necessary to clarify the entire population structure of Atlantic swordfish.

RÉSUMÉ


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qu’il est possible que des échanges se produisent avec les espadons de la Méditerranée dans l’Atlantique Nord-Est, il est important de délimiter la zone de contact entre ces deux régions avant de comparer les échantillons de l’Atlantique Nord-Est et Nord-Ouest. A l’aide des données CR-I, nous montrons que la zone d’échange de l’Atlantique Nord-Est et de la Méditerranée se limite à une petite zone à l’ouest de Gibraltar, laquelle s’étend à l’ouest de 10°W. Les futures études portant sur la structure de la population dans l’Atlantique Nord devraient tenir compte de cet élément de preuve afin d’éviter des erreurs de Type I. Ce document souligne également d’autres domaines de recherche nécessaires en vue de clarifier la totalité de la structure de la population d’espadon de l’Atlantique.

RESUMEN

La estructura de población genética del pez espada del Atlántico se ha estudiado con datos de ADN mitocondrial (mtDNA) y ADN nuclear (nDNA) mediante varias metodologías de laboratorio y diversos enfoques analíticos. En este documento, se comparan los datos preliminares para los genes nucleares ldhA y calmodulina (Cam) del Atlántico sur con los datos publicados de otras regiones. Los resultados de Cam, pero no los de ldhA, confirman la división de las poblaciones de pez espada del Atlántico noroeste y el Atlántico sur. La distribución de los alelos Cam y los haplotipos CR-I es homogénea en todo el Atlántico sur (al sur de 5°N) y las comparaciones con el océano Índico confirman la diferenciación genética de estas dos cuencas. Sin embargo, el límite que separa el Atlántico norte del Atlántico sur no puede trazarse con los datos actuales y es necesario realizar un muestreo temporal-espacial adicional para resolver este tema. Es importante señalar que el Atlántico norte se ha descrito principalmente utilizando muestras del Atlántico noroeste y el Caribe, con una cobertura de muestreo muy limitada del Atlántico noreste. No obstante, dado que es probable que en el Atlántico noreste se produzca una mezcla con peces espada del Mediterráneo, es importante delimitar la zona de contacto entre estas dos regiones antes de comparar las muestras del Atlántico noreste y el Atlántico noroeste. Utilizando datos de CR-I, se muestra que la zona de mezcla del Atlántico noreste y el Mediterráneo está restringida a una pequeña zona al oeste de Gibraltar que se extiende hasta los 10°W. Estudios futuros de estructura de población en el Atlántico norte deberían considerar esta evidencia para evitar errores del Tipo I. Este documento resalta además otros campos de investigación necesarios para aclarar toda la estructura de población del pez espada del Atlántico.

KEYWORDS

Mitochondrial DNA, population genetics, stock identification, Xiphias gladius, Mediterranean, Atlantic

1. Introduction

For over a decade the genetic population structure of swordfish worldwide has been studied using mitochondrial DNA (mtDNA) (Alvarado Bremer 1992, 1994; Magoulas et al., 1993; Alvarado Bremer et al., 1995, 1996; Grijalva-Chon et al., 1993; Kotoulas et al., 1995; Rosel and Block 1996; Chow et al., 1997; Chow and Takeyama, 2000), allozymes (Grijalva-Chon et al., 1996; Pujolar et al., 2002) and nuclear DNA (Alvarado Bremer et al., submitted; Greig et al., 2000; Chow and Takeyama, 2000; Marques 2001; Nohara et al. 2003; Reeb et al., 2000, 2003; Ward et al. 2001) data. One of the most significant initial findings obtained with mtDNA data was the substantial differentiation of Mediterranean swordfish from Atlantic populations (Magoulas et al., 1993), and the lower levels of genetic variation of the Mediterranean population compared to any other population worldwide (Alvarado Bremer et al., 1995, 1996, 2005a; Rosel and Block, 1996; Pujolar et al., 2002). In addition, mtDNA data supported the inter-oceanic population differentiation of Atlantic, Mediterranean and Indo-Pacific swordfish (e.g., Alvarado Bremer et al., 1995, 1996, 2005b; Rosel and Block, 1996; Chow et al., 1997; Pujolar et al., 2002). Within the Atlantic, significant frequency differences between pooled North Atlantic samples and South Atlantic samples (South of 5°N) were demonstrated using both nucleotide sequence data (Alvarado Bremer et al., 1995, 1996, 2005b) and PCR-RFLP data (Alvarado Bremer et al., 1996; Chow et al., 1997) of the mtDNA D-loop or control region (CR). Phylogenetic interpretations of these mitochondrial data
(Alvarado Bremer et al., 1996. 2005a and b) indicate that the signal that differentiates Northwest Atlantic and the South Atlantic populations is explained in part by the heterogeneous distribution of mtDNA lineages belonging to two highly divergent clades (Alvarado Bremer et al., 1995). Clade II haplotypes (theta subgroup), which have not been detected in the Indo-Pacific (Alvarado Bremer et al., 1996; 1998a; Rosel and Block, 1996; Reeb et al., 2000), occur at a low frequency in the South Atlantic, increase in abundance towards the North Atlantic, and reach their highest frequency in the Mediterranean (Alvarado Bremer et al., 1995, 1996, 2005a and b). In addition, differences between NW Atlantic and South Atlantic swordfish populations can be explained by the heterogeneous distribution of lineages belonging to Clade I (Alvarado Bremer et al., 1996, 1998a, 2005b). Clade I haplotypes can be assigned into two groups of CR-I haplotypes; the alpha lineages that contain a nucleotide polymorphism that determines the presence of an Rsal restriction site that rarely occurs (<0.05%) among non-alpha lineages, and the beta lineages that lack this restriction site. Beta-lineages, which comprise the majority (90-95%) of Indo-Pacific fish, are more common in the South Atlantic than in the NW Atlantic and the Mediterranean (Alvarado Bremer, 2005b).

On the basis of the phylogeographic association of mtDNA lineages, Alvarado Bremer et al. (2005a) speculated that Clade I originated in the Pacific whereas Clade II originated in the Atlantic. The co-occurrence of these highly divergent clades in the Atlantic appears to be the consequence of historical unidirectional gene flow of swordfish from the Indo-Pacific into the South Atlantic. An analogous phylogeographic association of to clades has been reported in several other pelagic fishes (Finnerty and Block, 1992; Graves and McDowell, 1995; Alvarado Bremer et al., 1998; Graves, 1998; Chow et al., 2000) and characteristic of other cosmopolitan pelagic fauna (Perrin et al., 1978; Bangma 2006). In addition the higher proportion in the S. Atlantic of the ubiquitous beta-lineages, compared to the NW Atlantic gives additional support to this interpretation (Alvarado Bremer et al., 1996, 2005a and b).

The analysis of several nuclear loci is concordant with the mtDNA heterogeneity that differentiates NW Atlantic from South Atlantic swordfish. These studies include the analyses of aldolase B (aldB) and lactate dehydrogenase A (ldhA) (Greig et al., 1999; 2000), and the PCR-RFLP analysis of the nuclear locus calmodulin (CaM) gene intron-4 (Chow and Takeyama, 2000). However, in spite of the considerable advance in our understanding of Atlantic swordfish genetic population structure, it should be noted that both mtDNA and nDNA studies share several shortcomings. First, the North Atlantic has been characterized primarily by NW Atlantic and Caribbean samples, with very limited sampling coverage of the NE Atlantic. Accordingly the genetic homogeneity within the North Atlantic (East versus West) has not been tested. However, because Mediterranean swordfish potentially mix with NE Atlantic swordfish, the extent of this admixture has to be determined first to prevent the inclusion of mixed samples that may lead to Type I errors. Similarly, the boundaries (temporal and/or geographic) and the possible admixture of North and South Atlantic subpopulations remain to be resolved. Again, before comparing these two regions the extent of admixture of the South Atlantic swordfish with Indian Ocean swordfish has to be resolved. This study explores some of these questions by presenting additional sampling coverage from several regions of the Atlantic Ocean, while at the same time underlines future areas of research needed to further clarify the population structure of Atlantic swordfish.

2. Material and methods

2.1 Samples

Swordfish tissue (skeletal muscle) was obtained from commercial fishery operations through observer programs of the following countries: Brazil, United States, South Africa and Spain. For ldhA data included three South Atlantic swordfish samples collected off Brazil (Table 1) and the pooled NW Atlantic sample included in Greig (2000). Sampling details for these two regions are given in Marques (2002) and Greig (2000), respectively. For mtDNA, samples comparing the contact between the Indian Ocean and the South Atlantic included Namibia Banks (n= 34), Agulhas Bank (n= 29), and Mozambique Channel (n=24) (Figure 4). Details for additional samples are given in Alvarado Bremer et al. (1998a) and included swordfish from the Northeast Atlantic (n=60), Gulf of Guinea (n=84), and the tropical western Indian Ocean (n=45). Analysis of the CaM locus included samples from Namibia Banks (n=28), Agulhas Banks (n=30), which were compared against the NW Atlantic, South Atlantic and Indian Ocean samples of Chow and Takeyama (2000).

2.2 Laboratory procedures and statistical analyses

Procedures for DNA extraction and PCR amplification of the mtDNA control region I (CR-I) are described in detail in Alvarado Bremer et al. (1996) and in Alvarado Bremer et al. (2005b, and references therein). The
3. Results

3.1 Mitochondrial data

The extent of contact between Indian Ocean and South Atlantic swordfish was evaluated with an AMOVA (Excoffier et al., 1992) that compared samples from Agulhas Banks, the Mozambique Channel, tropical Indian Ocean and eastern South Atlantic waters (Namibia Banks). Corroborating previous studies, South Atlantic and Indian Ocean swordfish were significantly different from each other. This differentiation can be explained by two patterns. First, the phylogeographic association of mtDNA lineages confirms that Clade II lineages are absent from the Indian Ocean, but occur in Namibian waters at a frequency (4%) similar to those in Brazil-Uruguay (4%) and the Gulf of Guinea and (6%) (Alvarado Bremer et al., 1995b; 2005). Second, the proportion of Clade I alpha (RsaI) lineages in Namibia (50%) is not different from that reported by Alvarado Bremer et al (2005b) for the Gulf of Guinea (45%) and Brazil-Uruguay (45%), but significantly higher (P<0.01) than the frequency of the pooled Indian Ocean samples (11%) reported in this study (Figure 1).

The differentiation between NE Atlantic and Mediterranean swordfish was examined with a hierarchical analysis of variance (Holsinger and Mason-Gamer, 1996). The results of this analysis indicates that Atlantic samples collected west of the 10°W, are genetically differentiated from Mediterranean samples and display temporal stability among the years compared (Figure 2). Furthermore, the comparison of Clade II lineages that include reciprocally monophyletic theta-Atlantic and theta-Mediterranean (Alvarado Bremer et al. 2005b), suggest that haplotypes of Mediterranean origin in these waters (Figure 3) may be absent or very rare.

3.2 Nuclear Calmodulin locus

The relative frequencies of Cam locus alleles A and B are included in Figure 4. Allele A was the most common allele in both Namibia (85.7%) and Cape Town (86.6%). These frequencies are very similar to those reported by Chow and Takeyama (2000) for two other South Atlantic samples, namely the Gulf of Guinea (84%) and Brazil (88.7%) which contrast with the frequency reported by these authors for the NW Atlantic (49.3%). In consequence, the allele frequency distribution in the entire South Atlantic appears to be very homogeneous in agreement with the mtDNA CR-I data presented in this study (Figure 1). In addition, the South Atlantic and the NW Atlantic were significantly different (P<0.01). Similarly, the comparison of the pooled South Atlantic sample against the pooled Indian Ocean sample, where allele A is nearly fixed (99.9%) (Chow and Takeyama 2000), was also significantly different (P<0.05).

3.3 Nuclear ldhA locus

The allele frequencies at this locus were heterogeneous among the three samples collected off Brazil (Figure 5). In addition, Trip 2 was not in HWE. The allele frequency of Trip 3, the southermost of the three Brazilian samples, was not significantly different (P=0.0347) from the pooled NW Atlantic sample (n=173), with alleles 2 and 4 each occurring at about 6%. By contrast, the respective frequency of these two alleles in Trip 1 is about 1% whereas in Trip 2 allele 2 was found at 2% and allele 4 was not detected.

4. Discussion

4.1 Contact between the South Atlantic and Indian Ocean-Cam data

The Cam locus is monomorphic for allele A in the Pacific and nearly fixed for this allele in the Indian Ocean (99.93%), whereas in the Atlantic the frequency of allele A decreases towards the NW Atlantic, where allele B reaches its highest frequency (51%). A similar pattern has been described by the global relative frequency of mtDNA clades I and II (Alvarado Bremer et al. 2005a), and also within Clade I by the global relative frequency of RsaI alpha lineages relative to the ubiquitous beta lineages, of putative Pacific origin (Figure 1). The frequency of Beta lineages is highest in the Pacific, followed by the South Atlantic and lowest in the NW
Atlantic. Thus both mtDNA and Cam data are concordant with the interpretation of historical uni-directional gene flow from the Indo-Pacific into the South Atlantic (Alvarado Bremer et al. 2005 a and b).

4.2 Contact between northeast Atlantic and Mediterranean

The significant mtDNA differentiation of swordfish samples from the western Mediterranean (East of Gibraltar) against NE Atlantic samples west of 10°W, suggest that mixing of this two populations is limited to the region west of the strait of Gibraltar extending to 10°W and between 30-40°N (Figure 3). However, in a separate study (Viñas et al. 2007, in this volume) suggests that Mediterranean swordfish may invade NE Atlantic waters beyond 10°W, although these movements appear to be restricted to the coastal waters off Morocco as far south as 20°N. By contrast, the movement of Atlantic fish into the Mediterranean appears to be extremely rare, as no Atlantic Clade II (theta-Atl) lineages have been detected in that basin (Alvarado Bremer et al. 1999; this study). The demarcation of the region of contact between Atlantic and Mediterranean populations is extremely important for future studies attempting to resolve the genetic population structure of swordfish in the North Atlantic. Accordingly, when testing the null hypothesis of panmixia in the North Atlantic by comparing samples from the NE and the NW Atlantic, it would be prudent to exclude from the NE Atlantic region samples collected east of 10°W and between 25-40°N to prevent Type I errors due to the presence of Mediterranean fish.

4.3 Concordance in differentiation of northwest Atlantic and South Atlantic swordfish populations

The pattern of differentiation among swordfish populations revealed by the Cam locus and the mtDNA CR-I data is concordant at four levels, and include both ancestral and on-going patterns of gene-flow. First, both loci reveal significant differentiation among Indo-Pacific, South Atlantic and NW Atlantic basins. Second, no genetic heterogeneity is detected among South Atlantic samples. Third, both Cam and mtDNA support the inferred pattern of unidirectional historical gene flow from the Indo-Pacific into the Atlantic around southern Africa facilitated by the Agulhas current. Finally, data for these two loci suggest that current migration between adjacent regions, Indian-South Atlantic, and South Atlantic-NW Atlantic must be limited. Such concordance could not be corroborated with ldhA data. Previously, ldhA data had revealed inter-oceanic differentiation among NW Atlantic, Mediterranean and Pacific samples (Greig, 2000; Greig et al. 1999, 2000) concordant with mtDNA data. However, the comparisons of Brazilian samples against NW Atlantic failed to detect differences between these two regions (Figure 5). It should be noted that the samples assayed with mtDNA, ldhA and Cam were not the same. Accordingly, it would be desirable to characterize the same samples with the complete set of informative loci in order to exclude the possibility that the observed differences or similarities are due to sampling errors.

4.4 Future studies

Future analysis of Atlantic swordfish should include additional loci, including microsatellite data and additional exon-primed-amplified-introns. Of particular concern is to characterize the temporal and/or spatial differentiation of populations, as well as the levels of gene flow among adjacent populations. However, before these analyses are conducted, it is important to re-examine the patterns of distribution and abundance of swordfish throughout the Atlantic derived from fishery data to determine the relevance of characterizing genetically putative areas of contact. Accordingly, areas of contact may be punctuated and may occur in areas where the abundance of swordfish is low, and where gene flow may not be taking place (e.g., feeding areas). Finally, while differences between the NW Atlantic and South Atlantic swordfish populations appears to be clear, additional data from NE Atlantic waters is needed to determine the extent of differentiation of swordfish in these waters relative to other Atlantic regions.

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References


Table 1. Capture data for South Atlantic samples characterized with \textit{ldhA} (from Márquez, 2001).

<table>
<thead>
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<th>Trip</th>
<th>Capture date</th>
<th>Latitude; Longitude</th>
<th>n</th>
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<td>50</td>
</tr>
<tr>
<td>2</td>
<td>March 2000</td>
<td>0º-2ºN; 35º-36ºW</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>May 2000</td>
<td>8º35'S-14º09'S; 31º16'-31º24'W</td>
<td>35</td>
</tr>
</tbody>
</table>

Figure 1. Map depicting the relative frequency of mtDNA lineages grouped as described in Alvarado Bremer \textit{et al.} (1996, 2005b).
Figure 2. Hierarchical analysis of nucleotide diversity contrasting the patterns of variation among the Mediterranean samples (Alboran and Balearic Islands) and the NE Atlantic Iberian samples collected west of Gibraltar (IBE$n$), with samples separated within region by swordfish mtDNA clade (I or II). Acronyms for the Iberian samples include the longitude followed by the collection years.
Figure 3. Minimum spanning trees depicting the differential distribution of Clade II lineages in the area adjacent to Gibraltar. Circles represent specific lineages present in the sample with the length of the lines that connect them representing the number of mutational steps between haplotypes. Filled circles represent the presence of a lineage in the particular geographical area where the tree is superimposed, whereas empty circles the absence of the lineage. The diameter of each circle represents the frequency of that particular lineage in each sample.
Figure 4. Relative frequency of Cam locus alleles A and B. Samples characterized in this study were Namibia and Agulhas Banks (southern tip of Africa). Data for the NW Atlantic, Brazil, Gulf of Guinea and Indian Ocean are from Chow and Takeyama (2000).
Figure 5. Distribution of *ldhA* lineages in the Atlantic. The South Atlantic samples (South of 5°N) are from Marquez (2001) and the NW Atlantic sample is from Greig (2000).