

THE USEFULNESS OF MITOCHONDRIAL DNA STUDIES TO DEFINE MANAGEMENT UNITS OF THE SWORDFISH, *XIPHIAS GLADIUS* : A REVIEW OF CURRENT LITERATURE

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The swordfish, *Xiphias gladius*, is the target of a large, multi-million dollar fishery with world catches exceeding 77 thousand metric tons in 1990 (Food and Agriculture Organization, 1992). Swordfish catch greatly outnumbers that of all other billfish combined. A steady decrease in the size of swordfish landings along with a significant decline in catch per unit effort in both the Atlantic Ocean and Mediterranean Sea (Berkeley, 1989) promoted measures to be adopted by the International Commission for the Conservation of Atlantic Tunas (ICCAT) member nations to reduce fishing effort in the Atlantic. Consequently, U.S. landings of Pacific swordfish increased dramatically a few years later (Boggs and Ito, 1993) as fleets moved into this new fishery. Because little information was available that could delineate the geographic distribution of biological stocks, fishery biologist turned to molecular techniques with the hope of identifying genetically distinct units to assist the management of the fishery.

At this time, there are seven papers and a number of abstracts published on the geographic distribution of mitochondrial DNA (mtDNA) in swordfish (Alvarado-Bremer *et al.*, 1992; 1995; in press; Margoulas *et al.*, 1993; Grijalva-Chon *et al.*, 1995; Koutoulas *et al.*, 1995; and Rosel and Block, 1996). MtDNA has been preferred as a first approach to examining the geographic distribution of genetic variation because the techniques are easy to use and the data analysis is often straightforward. Phylogenetic trees are usually drawn to show whether two populations are monophyletic with respect to one another indicating genetic isolation between the two. In the case of continuously distributed species with the potential to disperse great distances during some stage in their life history, a tree showing reciprocal monophyly between two geographic locales makes a very strong case for population subdivision and underscores the importance of a species' history in shaping the geographic distribution and levels of genetic diversity we see in contemporary populations (e.g. Reeb and Avise, 1990).

A common theme in all the mtDNA studies of swordfish listed above is that this species is composed of two monophyletic clades (Clade I and II). These clades are not equally distributed in all oceans, however they are also not highly localized to a single geographic area which complicates the use of a phylogeny. Clade II is found in highest frequency in the Mediterranean and northern Atlantic while Clade I is distributed globally (including the Mediterranean). To date, there has been no record of Clade II in the Pacific. An analysis of molecular variance reveals significant geographic partitioning of molecular variation among the three ocean basins, indicating that swordfish populations are structured on a global scale (Rosel and Block, 1996). MtDNA cannot tell us why these two clades diverged but estimates of genetic divergence between the two clades, and estimates of the rate of sequence evolution with the mtDNA genome of vertebrates suggest that this separation took place in the mid to late Pleistocene, a time characterized by a dramatic fluctuation between warm and cold climates. There is general agreement amongst the papers published that Atlantic, Pacific, and Mediterranean populations represent separate stocks. To extrapolate more information on genetic stock structure within ocean basins, researchers realized that mtDNA presented a serious problem.

The difficulty of using mtDNA for discerning a greater level of swordfish stock structure lies in the fact that among swordfish, the genetic diversity within the mtDNA genome is very recently derived. This is supported by the inability of mitochondrial markers other than the highly variable mitochondrial control region to exhibit sufficient

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polymorphism (e.g. Alvarado Bremer *et al.*, 1995, in press; Rosel and Block, 1996). Evidence of this problem is also apparent with the observation that nearly every mutation in the mitochondrial control region is the result of a transition, a rapidly occurring class of nucleotide base substitutions in which a purine is replaced by another purine or a pyrimidine is replaced by a different pyrimidine, but never is a pyrimidine converted to a purine (or vice versa). For a typical vertebrate control region, transitions outnumber transversions as much as ten to one so the older two taxa are, the more likely transversions will accumulate between them. The paucity of transversions suggests that swordfish populations sampled in the studies to date are quite young. Compounding this problem of recently derived mutations, is the fact that some of these transitions have experienced back mutation (homoplasy). Normally, phylogenetic programs can correct for this back mutation by referencing transversions in the data. However, the swordfish populations fall in an historical time frame in which there has not been enough time for sufficient transversions to accumulate. High levels of homoplasy can be a seriously hinder the accuracy of phylogenetic trees. For this reason, interpreting the mtDNA data to date beyond the message that there is stock structure at an ocean basin scale, is not recommended.

When commonly employed parameters are used to analyze the swordfish data, the resulting phylogenetic tree strongly supports the view that modern swordfish populations trace a common ancestry to two mtDNA lineages that lived during the Pleistocene. One of these clades (I) appears to have underwent an expansion out of the Atlantic and into the Pacific (Reeb *et al.*, 1996). However, in a recent analysis by Reeb and Block (unpublished results) of a combined set from Rosel and Block (1996) and Avarado-Bremer *et al.* (1995), the phylogenetic tree does not statistically support (using the bootstrap technique) the geographic partitioning of discrete clusters of closely-related individuals within clades I and II. This result is in contrast to findings reported in Alvarado Bremer *et al.* (1996, in press) which reports discerning an additional clade within Clade I giving rise to the interpretation that North and South Atlantic populations are different. We do not agree with the statistical analysis of their tree which uses a method that determines whether a branch length is significantly greater than zero as opposed to sub-sampling the data 100 times (bootstrapping) and asking how many times we get the same tree topology. Because of the error associated with estimating branch lengths caused by alignment, differential weighting of transitions and transversions (of which their 2:1 ratio is incorrect), as well as counting insertions or deletions of multiple bases adjacent to one another as multiple mutations, we believe the test of significance in branch lengths used by Alvarado Bremer *et al.* is invalid. Because bootstrapping is most commonly employed to test the significance of trees, and because the bootstrap values for the swordfish data set were so low (ranging from 1 to 20% when only values above 50% are ever shown on a tree), we are surprised that Avarado Bremer *et al.* would seriously advocate an additional clade.

A very similar controversy has been observed in our own human species. Phylogenetic analysis of first, mtDNA and later, nuclear genes, is consistent with the hypothesis that a small population from Africa underwent a rapid population expansion moving out of Africa multiple times during the late Pleistocene to colonize the globe (e.g. Cann *et al.* 1987; Vigilant *et al.*, 1991; Tishkoff *et al.* 1996). However, due to the multiple colonization events and the recent divergence, phylogenetic trees of humans

have limited utility in showing monophyletic clustering of individuals belonging to a single geographic population. The better approach at showing population divergence, especially with mtDNA, has been with acquiring a very large sample size and then documenting significant differences in allele frequencies or showing a significant difference in the geographic partitioning of genetic variance (e.g. the WINAMOVA program of Excoffier *et al.*, 1992). In spite of the tremendous amount of money and effort afforded to the study of human evolution, there is still a group of population geneticists and anthropologists who do not fully accept the "Out of Africa" hypothesis and point to the need for yet more molecular evidence to better refine and statistically support this scenario (Ayala, 1995). In addition, there are some anthropologists who refuse to accept molecular evidence as useful in anyway.

Because of the nature of evolution in the mitochondrial genome, and the recent, highly vagile history of human populations, this debate over these early data sets will continue for some time. However, now that mtDNA has provided us novel insights into understanding human history, we know better how to choose molecular markers. We know that we need loci, such as microsatellite loci, that evolve faster than mtDNA. We also realize that we need multiple nuclear markers which track the genetic contributions of both males and females, not just females as in mtDNA. The development of a Y chromosome male marker (Hammer, 1995) has also been useful to support the mtDNA findings for an African origin of humans. And we also need to know a rate and mechanism of sequence evolution for these markers specifically for the species we study so that we can more accurately estimate the level of diversity, genetic divergence, and the length of branches on phylogenetic trees. This has yet to be published for perciform fishes although our lab is currently working on this problem.

In summary, the amount of information gathered from seven molecular studies published on the broadbill swordfish *Xiphias gladius* has brought us a long way in a short amount of time, to a point not that far from where our understanding of human evolution (which is better funded and better studied) has come to lie. It is to the advantage of those studying swordfish (in fact, all pelagic fish) population genetics to interact more with human researchers and to present our work in forums such that it will be scrutinized by those who understand the complications involved with genetic analysis of closely-related populations. Those who provide support of genetic studies of pelagic fishes should realize that there is no simple solution to managing a species with the ability to disperse great distances. Clearly, genetic studies will not give us the ultimate answer, but then nothing else has either. We know now based on molecular genetics that, at the very least, swordfish should be managed on an ocean basin scale. For ICCAT managers, genetics has provided remarkable evidence based on the clear partitioning of swordfish into two clades that there most exist separate breeding populations. The two populations are mixing within both the Atlantic and the Mediterranean. The higher frequency of clade II swordfish within the Mediterranean leads to the suggestion that there exists within the Mediterranean a breeding ground for clade II swordfish. Maintaining this genetic diversity requires management which recognizes that the two units exist.

Even if further molecular genetics does show a significant difference between two geographic locales within the same basin, where in the ocean would one draw the boundary separating one management unit from the other if we know that occasionally, individuals stray as the genetic evidence suggests? We advocate a combined study of

genetics and archival/ satellite tagging as an approach that needs to be considered a priority. From this, we can gather both historical (from genetics) and contemporary (from tagging) patterns of gene flow from which management strategies can be devised. We are seeking funds for such a study to discern in the Atlantic where clade II versus clade I swordfish feed and breed. Thus, the management unit should contain a genetic basis but include a probability of dispersal into a new unit over time based on emigration from tagging studies. In addition, fishery biologists have yet to define over what time scales these pelagic populations are temporally and spatially stable, a necessary component to management. Amongst the most remarkable result from all of the genetic studies to date on swordfish is the apparent lack of genetic diversity maintained in swordfish populations prior to the late Pleistocene (300,000 years ago). This indicates that these populations may not be stable over long time frames. We are concerned with why this occurs and swordfish managers should recognize that the species they are working with has less genetic diversity than human populations which have been on earth for a much shorter period of time than *Xiphias gladius*.

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