Mitochondrial DNA Genetic Similarity of Atlantic and Pacific Skipjack Tuna and Its Management Implications

JOHN E. GRAVES AND ANDREW E. DIZON

Southwest Fisheries Center, National Marine Fisheries Service, NOAA, La Jolla, California, U.S.A.

Restriction enzyme analysis of mitochondrial DNA (mtDNA) provides the most powerful, practical tool currently available for determining the genetic basis of population structure. This technique applied to skipjack tuna from the Atlantic and Pacific Oceans demonstrated no significant genetic differentiation between fish from different ocean basins although within-sample variation was detected. These results are consistent with the lack of morphological and electrophoretic differentiation of Atlantic and Pacific skipjack tuna found in previous studies and strongly suggest sufficient gene flow between ocean basins to prevent genetic differentiation. As no significant genetic differentiation was exhibited between Atlantic and Pacific skipjack tuna it is unlikely that genetically distinct stocks exist within the Atlantic Ocean. Consequently, any subdivisions of Atlantic skipjack into management units will have to be based upon non-genetic criteria.

L'analyse de l'ADN mitochondrial (ADNmt) par les enzymes restrictifs est la méthode la plus efficace et pratique actuellement disponible pour déterminer la base génétique de la structure de la population. Cette technique, appliquée au listao des océans Atlantique et Pacifique, n'a pas montré de différenciation génétique significative entre les poissons de différents bassins océaniques, mais une variation a été décelée entre les échantillons. Ces résultats concordent avec le manque de différenciation morphologique et électrophorétique entre listaos de l'Atlantique et listaos du Pacifique qui a été observée dans les études antérieures, et donnent de fortes raisons de penser que l'échange de gènes entre les bassins océaniques est suffisant pour empêcher une différenciation génétique. Aucune différenciation de cette nature n'ayant été observée entre les listaos de l'Atlantique et ceux du Pacifique, il est peu probable qu'il existe dans l'Atlantique des stocks distincts du point de vue génétique. Toute subdivision du listao de l'Atlantique en unités de gestion devra donc se baser sur des critères non-génétiques.

El análisis del DNA mitocondrial (mtDNA) empleando enzimas restrictivas es en la actualidad el método más adecuado y práctico para determinar la base genética de la estructura de la población. Esta técnica, aplicada al listado del Atlántico y del Pacífico, demostró que no había una diferenciación genética importante entre los peces de los dos océanos, si bien se observó variación entre las muestras. Estos resultados concuerdan con la falta de diferenciación morfológica y electroforética del listado del Pacífico y del Atlántico, encontrada en anteriores estudios y sugiere categóricamente la existencia de una corriente genética. Al no encontrarse esta diferenciación entre el Atlántico y el Pacífico, es poco probable que existan stocks genéticamente distintos dentro del Atlántico. Por tanto, cualquier subdivisión del listado atlántico en unidades de ordenación deberá estar basada en criterios no-genéticos.

1. Introduction

The concept of the unit stock is fundamental to fisheries biology. Clearly, if all exploited fish species were composed of genetically isolated, intraspecific groups which were self-sustaining and independently vulnerable to the effects of fishing, fisheries management would be considerably simplified. However, as there are almost as many different types of underlying population structures as there are exploited species, it is unrealistic to assume that the unit stock concept is applicable to all fisheries.

Exploited fish species have intraspecific population structures that fall along a continuum from species with discrete unit stocks to panmictic species which demonstrate no intraspecific population structure at all (random mating). For example, the Atlantic herring displays an intraspecific population structure close to the unit stock concept (Iles and Sinclair 1982), whereas some pelagic fishes (sauries, billfishes) may almost be panmictic. To effectively manage a fishery one must have a clear understanding of the population structure of the exploited species.

The nature and amount of gene flow within and between intraspecific groups determines population structure. However, because gene flow is difficult to measure most fisheries investigators of population structure search for intraspecific genetic differentiation which results from an absence or severe reduction of gene flow. Thus, genetic differentiation, or more properly, the phenotypic expression of genetic differentiation, has provided the basis for most fisheries analyses of population structure.

Phenotypic characters used to distinguish intraspecific populations must have a strong genetic basis. Many characters typically used to differentiate populations or stocks, such as morphometrics, meristics, reproductive features (spawning season, fecundity), growth rates, etc., may have a strong genetic basis — yet their expression can be strongly influenced by the environment. A large environmental influence on any character used to infer genetic relationships can lead to spurious conclusions. For example, fish of different genetic and geographic origins living in cool water might have slower growth rates than those living in warmer water. An analysis of growth rates of these fish would indicate significant differences in growth rates but would provide no significant genetic information. Thus, caution must

be used when inferring genetic information from phenotypic data.

When discriminating or identifying stock units with phenotypic or genotypic characters, two criteria must be met: (1) the character used must show enough pooled intraspecific variation to allow discrimination of stocks and (2) there must be sufficient differentiation between stocks to allow identification with practical sample sizes. Some characters, though highly variable, will not differentiate stock units.

Much effort has been made to elucidate the genetic basis of population structure of skipjack tuna (*Katsuwonus pelamis*) using meristic, morphometric, growth, reproductive and electrophoretic characters (reviewed in Argue 1981). These studies have detected little character variation among skipjack tuna, and as a result have demonstrated only slight differentiation between individuals from different ocean basins. The small amount of intraspecific differentiation in genetic characters studied could either be attributed to strong selection resulting in the conservation of the analyzed characters or sufficient gene flow between ocean basins to prevent differentiation. To distinguish between the alternatives of character conservation or gene flow is difficult — one must search for additional characters with enough intraspecific variation to detect differentiation if it exists, or ultimately to accept the null hypothesis of no stock separation (gene flow between sampling locations).

This paper presents the results of a restriction endonuclease analysis of mitochondrial DNA (mtDNA) of skipjack tuna from the Atlantic and Pacific Oceans. Restriction enzyme analysis is currently the most powerful genetic technique available to population biologists for determining the genetic basis of intraspecific differentiation. This study was undertaken to determine if significant interocean genetic differences exist between skipjack, and to determine if further genetic analyses within the Atlantic Ocean are warranted.

2. mtDNA Analysis

The characters investigated in population analyses often differ greatly in the level and complexity of their underlying genetic basis as well as the degree to which they are susceptible to environmental influence. Most morphological, meristic, growth, and reproductive characters are the result of the interaction of several distinct genetic loci and some degree of environmental influence. Electrophoretic and immunogenetic characters are usually the products of one or a few genetic loci and are typically, but not completely, free from environmental influence. Only by investigating the actual genetic material (DNA) can one be sure of avoiding the problems of environmental modification.

The structure, function and evolution of the nuclear genome (nuclear DNA) are not well known. As a result, it is difficult to compare nuclear genomes of organisms for genetic studies. On the other hand, the mitochondrial genome (mtDNA) provides a very tractable molecule for comparative genetic analyses (Avise et al. 1979a). mtDNA is a small molecule and unlike nuclear DNA is maternally inherited and does not undergo recombination during meiosis (Giles et al. 1980). The complete nucleotide sequences of mouse, cow and human mtDNAs have recently been published (Bibb et al. 1981; Anderson et al. 1981, 1982). These and other sequence studies have demonstrated that while both the function and order of the mitochondrial genome are highly conserved in vertebrates, there appears to be considerable inter- and intraspecific variation of the nucleotide sequence (Brown et al. 1982).

Sequencing the mitochondrial genome is a costly and time consuming process which is currently not practical for population studies. However, the sequence of the mitochondrial genome can be investigated with restriction endonucleases, enzymes which recognize specific 4, 5 or 6 nucleotide base pairs on the DNA and consistently cleave (break) the DNA molecule at or near the recognition site. Nucleotide substitutions in the 4, 5 or 6 base pair recognition site result in the loss of the cleavage, and consequently, a reduction in the number of fragments (Figure 1). Similarly, base pair substitutions outside of the recognition site may create a new cleavage site and mtDNA fragment. After digestion with a restriction enzyme, mtDNA fragments are separated by size electrophoretically and the fragment patterns of the individual mtDNAs digested with the same restriction enzyme are compared. The loss or gain of a fragment (cleavage site) is considered to be the result of a single base pair substitution. Typically, several different restriction endonucleases are used in a population analysis and a minimum percentage mtDNA sequence divergence between individuals is calculated from the fragment patterns using established methods (Lansman et al. 1981; Nei and Tajima 1981).

mtDNA restriction enzyme analyses have demonstrated rapid nucleotide sequence evolution both at the inter- and intraspecific level. The rate of mtDNA evolution appears to be approximately ten times faster than that of single copy nuclear DNA (Brown et al. 1982). Results from studies within several classes of vertebrates have shown that individuals from the same population tend to have very similar mtDNA sequences while individuals from geographically disjunct populations exhibit some mtDNA sequence divergence (Avise et al. 1979a, b). These differences

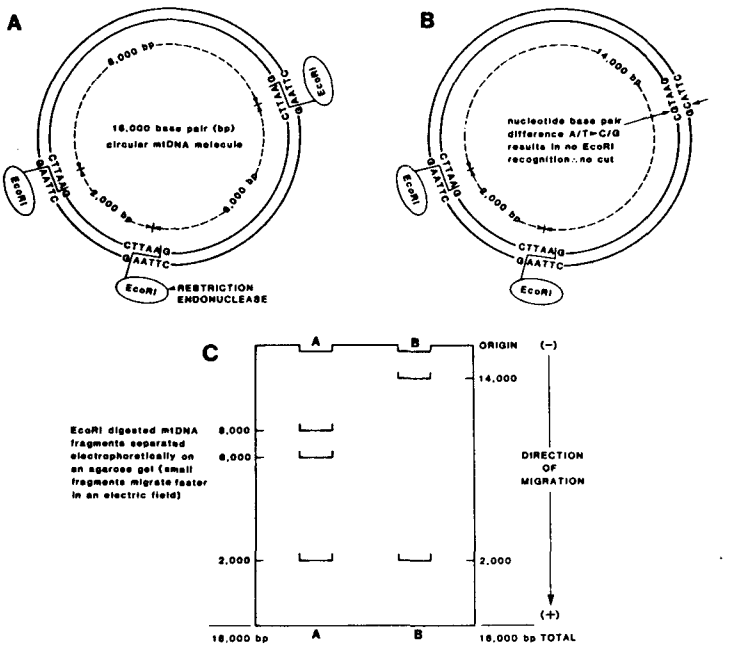

Figure 1. Restriction endonuclease analysis of mitochondrial DNA (mtDNA)

The mtDNA molecule is a double-stranded, circular chain of four nucleotide bases: adenosine (A), thymine (T), cytosine (C) and guanine (G). In the double-stranded DNA chain the nucleotide bases A and T align opposite each other on the two strands, as do C and G. Vertebrate mtDNA molecules consist of approximately 16,000 nucleotide base pairs. The information of the molecule is contained within the specific sequence of the nucleotide bases. Genetically similar individuals have similar mtDNA nucleotide sequences.

In our analysis of mtDNA of Atlantic and Pacific skipjack tuna we employed restriction endonucleases to compare the similarity of mtDNA sequences of fish from the two oceans. Restriction endonucleases are enzymes that recognize specific 4, 5 or 6 nucleotide base pair sequences of the DNA molecule and consistently cut (cleave) the DNA chain at or near the recognition site. Consequently, by comparing the mtDNA fragments of individuals after treatment with restriction endonucleases (digestion), we were able to infer differences or similarities in the mtDNA nucleotide sequence.

In this figure the restriction endonuclease EcoRI, which recognizes the nucleotide sequence GAATTC, encounters three sites to cleave the circular mtDNA of fish A (A). A genetic difference (base pair change) in the mtDNA of fish b has resulted in the loss of one EcoRI cleavage site (B). Consequently, digestion of mtDNA from these two fish with the restriction endonuclease EcoRI results in three mtDNA fragments from fish A and only two from fish B. The mtDNA fragments are separated by size on agarose gels with an electric current and visualized by direct staining or with radioactive labels (C). Genetically similar fish have similar restriction endonuclease digestion patterns while less related fish share fewer bands. Skipjack tuna from the Atlantic and Pacific Oceans have strikingly similar restriction endonuclease digestion patterns. This similarity strongly suggests that they have continued to have genetic contact (interbreed) since the uplift of the Panama land bridge.

become more pronounced at the subspecies and species level. The optimal resolution of mtDNA sequence differences occurs for populations which have been separated for 0.25 to 5 million years (Brown et al. 1982).

3. Results

In our laboratory at the Southwest Fisheries Center, National Marine Fisheries Service, we have applied restriction enzyme analysis of mtDNA to skipjack tuna from the Atlantic and Pacific Oceans (Dizon et al. 1983). Our investigation of seven Atlantic (one Ponce, Puerto Rico; six Rio de Janeiro, Brazil) and nine Pacific specimens (all from Honolulu, Hawaii) with nine restriction enzymes produced a total of 39 or 40 fragments per individual. Although intraspecific variation was demonstrated with four of the nine restriction enzymes (Table 1), none of the variants were consistently present in fish from one ocean and

not in the other. Thus, a genetic analysis which surveyed 1% of the skipjack mitochondrial genome demonstrated a mtDNA nucleotide sequence divergence of 0% between fish from the Atlantic and Pacific Oceans.

Table 1. Restriction endonucleases employed and the resultant patterns observed in skipjack tunas. Two types of analyses were performed: (1) mtDNA was purified and fragments were end-labelled with radioactive deoxynucleotides to enhance visualization of the gel. (2) Southern transfers of total genomic DNA were hybridized with a probe made from salmonid mtDNA. Abbreviations: HI = Hawaiian, PR = Puerto Rican and BRZ = Brazilian samples; numbers of individuals tested in parentheses; w = "wild" type (common pattern), var = "variant" type (mutated pattern).

	Restriction Patterns			
Enzyme type	HI (3 pooled) (purified mtDNA)	PR (1) (purified mtDNA)	HI (6) (Southern method)	BRZ (6) (Southern method)
Hind III	w	w	—	—
Hpa II	w	w	w	w
Mbo I	w	w	—	—
Pst I	w	var	w	w
Eco RI	w	w	—	—
Xba I	w	w	—	—
Sac I	w, var	—	w	w
Pvu II	w	—	w	w, var
Ava II	w, var	—	w	w

4. Discussion

As previously mentioned, for a genetic character to be a useful marker in population analyses, it must demonstrate sufficient pooled intraspecific variation to allow stock unit differentiation. Four of the nine restriction enzymes employed in this study showed intraspecific variation. Although there are presently no data from other fishes with which to compare our results, they can be compared with results from mammals surveyed with the same restriction enzymes. mtDNA restriction analysis of ten common chimpanzees with five restriction enzymes produced a total of ten variants (Ferris et al. 1981) while eight variants were found in sixteen skipjack tuna with the same enzymes. Thus skipjack tuna appear to be about one-half as variable as chimpanzees. Relative to population analyses of chimpanzees and other mammals, it would appear that sufficient mtDNA variation is exhibited by skipjack tuna to differentiate major intraspecific groups if they exist (Avise et al. 1979a, b; Lansman et al. 1981).

In the vertebrates studied to date, mtDNA appears to evolve at a rate of approximately 0.015 substitutions per nucleotide base pair per million years (Brown et al. 1982). If skipjack tuna of the Atlantic and Pacific Oceans have been separated since the uplift of the Panama land bridge some 3.1 million years ago (Keigwin 1978, 1982), one would expect a nucleotide sequence divergence of about 4–5%. Our analysis of 169 nucleotide base pairs deomonstrated a mtDNA sequence divergence of 0%. Even if a fragment were found to be present in Atlantic skipjack tuna and absent in Pacific fish with the use of one additional restriction enzyme, the sequence divergence between Atlantic and Pacific fish would only be 0.6%. It would therefore appear that skipjack tuna from the Atlantic and Pacific Oceans have not been genetically isolated since the rise of the Isthmus of Panama.

This is not the first study to report considerable genetic similarity between Atlantic and Pacific skipjack tuna. In a series of electrophoretic and immunogenetic studies, Fujino and coworkers have demonstrated a high degree of genetic similarity between fish from the two oceans (Fujino and Kang 1968; Fujino 1970a; Fujino et al. 1981). These studies report slight allele frequency differences at a few genetic loci which can be used to distinguish Atlantic and Pacific skipjack tuna providing one has large samples. However, no electrophoretic characters were found to be fixed for different alleles in fish from the two oceans; hence, it is not possible to differentiate individual fish.

The magnitude of the electrophoretic and immunogenetic differences between skipjack tuna from the Atlantic and Pacific Oceans is minute when compared to other conspecific fishes separated by the Isthmus of Panama. Tropical marine shorefishes on either side of the Panama land bridge, whether considered the same or very closely related species, exhibit between-ocean genetic distances an order of magnitude greater than those reported for skipjack tuna (Vawter et al. 1980). While 20–40% of the electrophoretic loci are fixed for different alleles between pairs of shorefishes on either coast of Panama, skipjack tuna exhibit no fixed allelic differences (Fujino 1970). It is significant that the dolphin fish (*Coryphaena hippurus*), a strictly pelagic species, is similar to the skipjack tuna in that no fixed allelic differences can characterize a fish as being from the Atlantic or Pacific Ocean and only slight allele frequency differences are found at a few loci (R. Rosenblatt and R. Waples, Scripps Institution of Oceanography, pers. com.).

The striking lack of genetic differentiation between Atlantic and Pacific skipjack tuna and dolphin fish, in contrast to the highly differentiated shorefishes, strongly suggests that there has been sufficient gene flow between ocean basins to prevent genetic differentiation of the pelagic species. The most plausible route for genetic interchange of tropical and subtropical, pelagic populations is around the Cape of Good Hope. Seasonal isotherms indicate that suitable water temperatures exist for migration of skipjack tuna and

other warm water pelagic species around the Cape of Good Hope throughout most of the year (Davies 1963). Furthermore, Japanese longline data (Silas and Pillai 1982) and South African commercial and sport fishery data indicate that skipjack tuna are seasonally abundant in waters around the Cape of Good Hope (S. Grant, Sea Fisheries Institute, South Africa, pers. com.).

Skipjack tuna are capable of swimming long distances. Although not long lived fish, tagging studies have shown some individuals to travel thousands of kilometres (reviewed in Argue 1981). If skipjack tuna are capable of crossing ocean basins as tag recovery data indicate, then it is most likely that they are also able to migrate between ocean basins. Furthermore, as skipjack tuna usually travel in large schools, the potential exists for significant gene flow between ocean basins.

Genetic differentiation can occur only if there is reproductive isolation between populations. Skipjack tuna do not have distinct spawning areas and behavioral isolation has not been reported. Ripe fish and larvae are found circumtropically in warm, pelagic waters (reviewed in Sund et al. 1981). Fish swimming across or between ocean basins can mate with fish from local or distant geographic areas. The lack of reproductive isolation of skipjack tuna populations increases the possibility for significant gene flow both within and between ocean basins.

The lack of significant mtDNA sequence differentiation between Atlantic and Pacific skipjack tuna is consistent with the natural history of the fish. The existence of suitable habitat for passage around the Cape of Good Hope, the strong swimming ability of the fish (Miyabe and Bard this volume; Argue et al., this volume), and the lack of discrete spawning areas (Cayré et Farrugio this volume) all promote gene flow within and between ocean basins. The effects of this gene flow are also reflected in the high degree of morphological and electrophoretic similarity of skipjack tuna from different ocean basins.

The purpose of this study was to apply a new, high resolution genetic technique to the problem of identifying the stock structure of skipjack tuna. The sixteen fish examined in this study demonstrated intraspecific variability of mtDNA restriction sites but did not display consistent sequence differentiation between ocean basins, a finding which is consistent with those of previous genetic studies. These results do not prove that all skipjack are genetically similar; rather, they demonstrate a high degree of genetic similarity between fish from the Atlantic and Pacific Oceans which suggests sufficient gene flow to prevent significant genetic differentiation. Although some intraspecific genetic differentiation may be uncovered as more of the mtDNA genome is surveyed (more nucleotide base pairs as opposed to more individuals), at this time there are no data to suggest that skipjack exist other than as a single, genetically homogeneous, circumtropical stock.

As significant genetic differentiation has not been demonstrated between Atlantic and Pacific skipjack tuna it is unlikely that marked differentiation exists between skipjack within the Atlantic Ocean. This does not exclude the possibility of dividing the skipjack tuna stock for management purposes into fishery units on other than genetic bases. However, if fishery management units are devised, they should be based on empirically derived exchange rates which should not be confused with true genetic stocks.