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Close genetic similarity of Atlantic and Pacific skipjack tuna (*Katsuwonus pelamis*) demonstrated with restriction endonuclease analysis of mitochondrial DNA

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Abstract

Restriction endonuclease analysis of mitochondrial DNA indicated a surprisingly high degree of genetic similarity between skipjack tuna (*Katsuwonus pelamis*) from the Atlantic and Pacific Oceans. The present results (1983) support the findings of previous morphological and electrophoretic studies. Evidently, since the uplift of the Panamá land bridge about 3.1 million years ago, there has been continued genetic contact between Atlantic and Pacific skipjack tuna, presumably via the Southern Ocean.

Introduction

Effective fishery management requires an understanding of the population or stock structure of the exploited organism. To investigate the genetic basis of stock structure, one must study genetic characters that demonstrate sufficient variation to permit discrimination of intraspecific groups or stocks. The genetic basis of population structure of skipjack tuna (*Katsuwonus pelamis*) has been reviewed by Argue (1981). From studies of meristic, morphometric and electrophoretic characters of skipjack tuna, geneticists have detected little character variation and have demonstrated only slight differentiation between individuals from different ocean basins. The apparent lack of significant differentiation in genetic characters could either be attributed to strong selection resulting in the conservation of the analyzed characters, or sufficient gene flow among ocean basins to prevent differentiation. To distinguish between the alternatives of character conservation or gene flow is difficult, since one must search for additional characters with enough intraspecific variation to detect differentiation if it exists, or ultimately accept the null hypothesis of gene flow.

Recent studies have reported high levels of inter- and intraspecific variation of mitochondrial DNA (mtDNA) sequences of several organisms (reviewed by Avise and Lansman, 1983). It appears that mtDNA evolves about an order of magnitude more rapidly than single-copy nuclear DNA (Brown *et al.*, 1982). Fragment pattern analyses of mtDNA from several mammalian species have shown that individuals from the same population typically possess very similar mtDNA sequences, while individuals from different populations exhibit some mtDNA sequence divergence (Avise and Lansman, 1983). Consequently, mtDNA sequence differences between populations can be determined with small sample sizes. Sequence differentiation of mtDNA becomes more pronounced at the subspecies and species level (Brown, 1983). The optimal resolution of mtDNA sequence differences occur for populations that have been separated for 0.25 to 5 million years (Brown *et al.*, 1982).

Unlike nuclear DNA, mtDNA appears to be maternally inherited and does not undergo recombination during meiosis in metazoans (Giles *et al.*, 1980). It exists as a closed circular molecule of 15 000 to 18 000 nucleotide base pairs which encode two ribosomal RNAs, 22 transfer RNAs and 13 messenger RNAs (reviewed by Brown, 1983). The complete nucleotide sequences of mouse, cow and human mtDNAs have recently been published (Anderson *et al.*, 1981, 1982; Bibb *et al.*, 1981). 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.

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 sampled with restriction endonucleases, enzymes which recognize specific 4, 5 or 6 nucleotide base pairs and consistently break the DNA within the recognition site. Nucleotide substitutions in the 4, 5 or 6 base-pair recognition site prevent endonuclease cleavage and thus reduce the number of mtDNA frag-

ments. Similarly, base-pair substitutions outside 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 individual mtDNAs digested with the same restriction enzyme are compared. The loss or gain of a fragment (cleavage site) is assumed to result from the substitution of a single base-pair. Typically, several different restriction endonucleases are used in a population analysis, and, from the fragment patterns, one calculates a minimum percentage mtDNA sequence divergence between individuals by established methods (Lansman *et al.*, 1981; Nei and Tajima, 1981).

Significant intraspecific variation of mtDNA sequences has been reported for several organisms and has permitted high-resolution investigations of the genetic basis of population structure (see Avise and Lansman, 1983). Studies that have compared results from (1) starch-gel electrophoresis of water-soluble proteins, and (2) from mtDNA restriction endonuclease analysis have demonstrated that the latter technique uncovers considerably more intraspecific variation and differentiation (Lansman *et al.*, 1983). Furthermore, because of the faster rate of mtDNA evolution, population-specific differences in mtDNA sequences accumulate more rapidly than electrophoretic differences, and significant genetic differentiation can be observed with smaller sample sizes (Lansman *et al.*, 1983).

The purpose of the present study was to examine the amount of variation in mtDNA sequence both within and between samples of Atlantic and Pacific skipjack tuna (*Katsuwonus pelamis*). This paper, which is the first study of intraspecific mtDNA variation in fishes, presents evidence of possible gene flow between skipjack tuna from the Atlantic and Pacific Oceans.

Materials and methods

Freshly caught and iced skipjack tuna (*Katsuwonus pelamis*) were purchased from fish markets in Honolulu, Hawaii ($n=9$) and Rio de Janeiro, Brazil ($n=6$), and a single specimen was purchased in Ponce, Puerto Rico. Livers and hearts were removed from the fish shortly after purchase and immediately frozen on dry ice. All samples were shipped on dry ice to the laboratory, where they were stored at −70 °C until processing. The study was performed in 1983.

Protocols for the purification and analysis of mtDNA used in this study are presented in Brown *et al.* (1979) and Lansman *et al.* (1981). Because yields of purified skipjack mtDNA from frozen tissue were low, it was necessary to endlabel mtDNA fragments with radioactive nucleotide triphosphates after restriction endonuclease digestion in order to visualize band patterns after electrophoresis (described by Lansman *et al.*, 1981). Three of the 9 Hawaiian skipjack (pooled to obtain sufficient purified mtDNA) and the single Puerto Rican skipjack were analyzed by this method.

Purification of mtDNA from contaminant nuclear DNA by density-gradient ultracentrifugation is possible only if the mtDNA remains in the denser, closed circular form. The low yields of mtDNA purified from the frozen skipjack tissues indicated that, due to freezing or handling, a large fraction of the mtDNA was not in the closed circular form. Consequently, a different method of analyzing mtDNA fragment patterns was followed for the 6 Brazilian and remaining 6 Hawaiian skipjack. For these specimens, total cellular DNA (mtDNA + nuclear DNA) was isolated, digested with restriction endonucleases, subjected to electrophoreses, and transferred to nitrocellulose filters [Southern (1975) method described by Lansman *et al.* (1981)]. Fragments of mtDNA were visualized against a background of nuclear fragments by hybridizing radioactively-labelled mtDNA purified from freshly sacrificed rainbow trout (*Salmo gairdneri*) to the skipjack mtDNA fragments. As only those fragments with high-sequence similarity will hybridize, association of labeled rainbow trout mtDNA with skipjack nuclear DNA fragments did not occur. Rainbow trout was chosen for a hybridization probe on the basis of availability. Hybridized fragment patterns were visualized autoradiographically.

The restriction endonucleases (Table 1) were purchased from New England Biochemicals and were used according to the manufacturer's directions.

Results and discussion

The 9 restriction endonucleases used in the endlabeling analysis of the mtDNA purified from the 3 pooled specimens of *Katsuwonus pelamis* from Hawaii and the single Puerto Rican specimen (Table 1) produced 25 and 26 fragments, respectively. Fragment patterns of 5 of the 9 restriction enzymes used in the endlabeling analysis were investigated in more detail in the southern (hybridization) analysis of the Brazilian and Hawaiian skipjack tuna. These 5 enzymes produced fragment patterns similar to those produced in the endlabeling analysis, and resolved an additional 14 fragments.

The size of the skipjack tuna mtDNA molecule (genome) was estimated by summing the mtDNA fragment sizes produced by a single restriction endonuclease. Fragment sizes of mtDNA were determined by comparison with the electrophoretic mobility of DNA fragments of known size (restriction enzyme digests of phage lambda DNA). The mean size of the skipjack tuna mtDNA genome, calculated from several digests with 5 restriction enzymes (*Hind* III, *Pst* I, *Sac* I, *Pvu* II and *Ava* II), was 16 900 base pairs for fish from both oceans. This value agrees well with the size of 16 700 base pairs reported for the mtDNA of 4 species of salmonids (Berg and Ferris, 1984) and is about 400 base pairs greater than the value reported for humans (Brown, 1981).

The number of base pairs examined in this study can be determined precisely. With the southern method, the

Table 1. Restriction endonucleases employed, number of base pairs recognized at the restriction (cleavage) site, and resultant patterns observed in the skipjack tuna *Katsuwonus pelamis*. Two types of analyses were performed: (a) mtDNA was purified and fragments were endlabeled with radioactive deoxynucleotide triphosphates to enhance visualization on the gel; (b) Southern transfers of total genomic DNA were hybridized with a probe made from salmon (*Salmo gairdneri*) mtDNA (see Ferris *et al.*, 1981). HI = Hawaiian, PR = Puerto Rican, and BRZ = Brazilian samples; numbers of individuals tested are given in parentheses; w = "wild" type (common pattern), var = "variant" type (mutated pattern). Dash = not examined

Enzyme type	Recognition sites (base pairs)	Restriction patterns			
		Endlabeled		Southern method	
		HI (3 pooled)	PR (1)	HI (6)	BRZ (6)
Hind III	6	w	w	–	–
Hpa II	4	w	w	w	w
Mbo I	4	w	w	–	–
Pst I	6	w	var	w	w
Eco RI	6	w	w	–	–
Xba I	6	w	w	–	–
Sac I	6	w, var	–	w	w
Pvu II	6	w	–	w	w, var
Ava II	5	w, var	–	w	w

restriction enzymes *Pst* I, *Sac* I and *Pvu* II, which recognize 6 base-pair sites (Table 1), made 2, 2 and 3 restrictions respectively, while *Ava* II which recognizes 5 base pairs, made 3 cleavages and *Hpa* II, a restriction enzyme which recognizes 4 base pairs, created 4 detectable fragments. Thus, a total of 73 base pairs was surveyed by the southern method. The restriction endonucleases used in the endlabeling method recognized an additional 96 base-pairs for a grand total of 169 base-pairs, about 1% of the total skipjack mtDNA genome.

Intraspecific variation in fragment patterns, reflecting variation in mtDNA nucleotide sequences, was demonstrated with 4 of the 9 restriction enzymes (Table 1). However, none of the fragments demonstrated consistent differences between fish from the two oceans. Three fragments were different between the Hawaiian skipjack tuna and the Puerto Rican specimen investigated in the endlabeling analysis, attributable to a single *Pst* I mutation in the Puerto Rican fish mtDNA. The other Atlantic skipjack tuna from Brazil had *Pst* I digestion patterns similar to those of the Hawaiian fish, and thus the Puerto Rican skipjack appears to be variant. Within-ocean variants were also found for digests with *Sac* I, *Pvu* II and *Ava* II (Table 1).

Currently there are no available data on intraspecific sequence variation in fishes with which to compare the results of this study. However, they can be compared with mammalian data obtained with the same restriction enzymes. Table 2 presents the mtDNA variation in common chimpanzees (*Pan troglodytes*) which is typical of other mammals (Ferris *et al.*, 1981). The chimpanzees exhibited 13 morphs for 10 individuals tested, whereas for

Table 2. Qualitative comparison of mtDNA variation in skipjack tunas (*Katsuwonus pelamis*) and in common chimpanzees (*Pan troglodytes*)

Tuna ($n = 16$)		Chimps ($n = 10$)[a]	
Enzyme type	No. of morphs	Enzyme type	No. of morphs
Hpa II	1	*Hpa* II	5
Pst I	2	*Pst* I	1
Sac I	2	*Sac* I	2
Pvu II	2	*Pvu* II	2
Ava II	2	*Ava* II	3
Total	9		13

[a] From Ferris *et al.* (1981)

the skipjack tuna only 9 morphs were discovered in 16 individuals examined. Thus, intraspecific mtDNA sequence variation in the skipjack tuna was less than in most mammals, although higher than levels reported for some mammals (Avise and Lansman, 1983); the variation in the tuna certainly appears sufficiently high to reveal significant intraspecific differentiation if it indeed exists.

The present study represents a limited investigation of mtDNA variation within and between Atlantic and Pacific skipjack tuna. The total number of base-pairs examined and the number of base-pairs examined per individual in this study are low compared with some recent mammalian studies. Furthermore, restriction enzymes which recognize 6 base-pairs were preferentially used in this study because they produce larger, more easily visualized fragments although they do not reveal as much variation as those which recognize 4 base-pairs. Even though a more extensive study of skipjack tuna will probably reveal more variants than did the present study (and perhaps even some population- or ocean-specific fragments), it is clear from these results that there is a striking lack of mtDNA differentiation between Atlantic and Pacific skipjack tuna.

Our results from a total sample of 7 Atlantic and 9 Pacific skipjack tuna demonstrate a sequence divergence of 0.0% between fish from the two oceans. Even if a restriction site had been found in fish from one ocean but not from the other, the difference would be only 1 substitution per 169 base-pairs or a sequence divergence of 0.6%. This value is very small compared to some mammalian disjunct conspecific populations which differ by about 3% (Avise and Lansman, 1983). If skipjack tuna from the Atlantic and Pacific Oceans have been separated since the uplift of the Panamá land bridge 3.1 million years ago (Keigwin, 1982) and their rate of mtDNA evolution is similar to other vertebrates studied, one would expect a sequence divergence of approximately 3% (Brown, 1983). Furthermore, this sequence divergence would be evident in each specimen sampled, and a sample size of $n = 1$ would be sufficient to demonstrate that a sequence difference existed.

Electrophoretic and immunogenetic studies of skipjack tuna have also reported a high degree of genetic similarity

between fish from the Atlantic and Pacific Oceans (Fujino and Kang, 1968; Fujino, 1970; Sharp and Pirages, 1978, Fujino *et al.*, 1981). Although some of these studies report small allele frequency differences at one or two loci between fish from the two oceans, no single character has been found which can distinguish an Atlantic from a Pacific skipjack tuna. This is in contrast to the large amount of genetic differentiation reported between conspecific and closely related (geminate) pairs of tropical marine shorefishes on either side of the Panamá land bridge which are genetically distinguishable at several different electrophoretic loci per pair (Vawter *et al.*, 1980). It is interesting to note that the dolphin fish *Coryphaena hippurus*, a strictly pelagic species, is similar to the skipjack tuna in that it demonstrates no significant interocean electrophoretic differentiation (R. Rosenblatt and R. Waples, personal communication).

The high degree of genetic similarity among individual skipjack tuna and among individual dolphin fish from the two oceans suggests that there is, or recently was, genetic contact between Atlantic and Pacific populations of these pelagic species. The most plausible route for genetic interchange is around the Cape of Good Hope. A seasonal coastal sport-fishery exists for skipjack tuna in South Africa, and Japanese longline data indicate that skipjack tuna have been caught off the Cape (Silas and Pillai, 1982). Seasonal isotherm patterns are consistent with the idea that skipjack tuna and other subtropical pelagic fishes can migrate around the Cape of Good Hope (Davies, 1963). Skipjack are strong swimmers and, although definite migrations have not been demonstrated, many tagged skipjack have been recovered thousands of kilometers from the site of first capture (reviewed by Argue, 1981). As skipjack often travel in large schools, the potential exists for significant gene flow around the Cape of Good Hope.

Genetic differentiation is promoted by reproductive isolation, a physical separation of populations, and a lack of migration between populations. Skipjack tuna do not have discrete spawning areas and larvae are found circumtropically in pelagic waters (Sund *et al.*, 1981). Furthermore, seasonal isotherm data and individual tag-return records indicate that skipjack tuna have the potential to migrate between ocean basins. Although it cannot be stated with any certainty just how much migration between ocean basins occurs, or has occurred in recent times, it would appear from the results of this study that there has been sufficient interchange to prevent genetic differentiation.

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