Mitochondrial DNA Sequence Similarity of Atlantic and Pacific Albacore Tuna (*Thunnus alalunga*)

John E. Graves[1] and Andrew E. Dizon

National Marine Fisheries Service, Southwest Fisheries Center, 8604 La Jolla Shores Drive, P.O. Box 271, La Jolla, CA 92038, USA

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Restriction endonuclease analysis of mitochondrial DNA purified from 11 south Atlantic (Capetown, South Africa) and 12 north Pacific (San Diego, USA) albacore tuna (*Thunnus alalunga*) revealed no restriction sites which could distinguish an Atlantic from a Pacific albacore. Although restriction site variation was found within the pooled sample, variants were found only in single fish. These results suggest either recent isolation of Atlantic and Pacific albacore or, more likely, at least a small amount of migration between the two ocean basins.

Une analyse par endonucléase de restriction de l'ADN mitochondrial purifié à partir de 11 thons (*Thunnus alalunga*) de l'Atlantique sud (Capetown, Afrique du Sud) et de 12 thons du Pacifique nord (San Diego, É.-U.) n'a révélé aucun site de restriction permettant de distinguer un thon de l'Atlantique d'un thon du Pacifique. Bien qu'on ait trouvé des variations dans les sites de restriction dans l'échantillon réuni, les variants ne se retrouvaient que chez des poissons isolés. Ces résultats indiquent soit un isolement récent des thons de l'Atlantique et du Pacifique ou, plus probablement, au moins une certaine migration entre les deux bassins océaniques.

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Fishery stocks, although not necessarily defined with genetic criteria, are usually assumed to have an underlying genetic basis (Gulland 1971). A stock boundary is indicated by significant differentiation of morphological, physiological, or biochemical characters between fish from different sampling sites and these characters are assumed to be under direct genetic control. Similarly, a marked change between geographic areas in catch per unit effort, mark and recapture returns, or other fishery statistics can also be used to define a stock boundary. A problem in fishery management arises, however, when fish from different putative stocks, defined on the basis of fishery statistic criteria, or fish from geographically distant areas cannot be distinguished with an analysis of genetic characters. The albacore (*Thunnus alalunga*) in the north Pacific Ocean presents such a problem.

Considerable effort has been expended on mark and recapture studies of albacore in the north Pacific Ocean to elucidate movement patterns. Basically, there appear to be two distinct groups of albacore which migrate to the western coast of North America. These groups differ in modal sizes of year classes, growth rates, movements, and areas of occurrence (Laurs and Lynn 1977; Laurs and Nishimoto 1979; Laurs and Wetherall 1981). Those fish which arrive at the California coast south of 40°N are slightly larger and have a faster growth rate than those which arrive at the coast north of 40°N (Laurs and Wetherall 1981). Furthermore, the northern group appears to migrate to the western north Pacific where it is vulnerable to the Japanese surface and subsurface fisheries, whereas the southern group tends to be recaptured in the central north Pacific. Although there is a slight interannual leakage between northern and southern groups (Laurs and Nishimoto 1979) the vast majority of recaptures of tagged fish has been from the area of marking. It is not known when fish leave the north Pacific surface fishery, but few mature adults are found in the temperate eastern Pacific, and spawning, as evidenced by ripe females and young larvae, seems mostly confined to the tropical central Pacific (Sund et al. 1981).

On the basis of mark and recapture data, modal sizes of year classes and growth rates of tagged fish, Brock (1943); Laurs and Lynn (1977); and Laurs and Wetherall (1981) have proposed that the two groups of albacore in the north Pacific represent distinct subpopulations. It is not known if fish from the putative northern and southern north Pacific albacore subpopulations remain reproductively isolated in the common spawning area or comprise genetically distinct stocks. To elucidate the genetic basis of population structure of the north Pacific albacore, an extensive genetic survey with considerable effort expended on sampling albacore on a large spatial and temporal scale will most likely be necessary. Before committing resources to such a task, we felt it would be beneficial to first determine the magnitude of genetic differentiation between albacore of different ocean basins. An estimate could then be made of the sampling effort required to detect genetic differentiation between the closely related putative subpopulations within the north Pacific.

In this paper we report that a genetic analysis of a small sample of albacore from Capetown, South Africa and San Diego, California employing restriction endonuclease analysis of mitochondrial DNA (mtDNA) revealed no significant genetic differentiation between albacore from the different ocean basins. This result is compared with other mtDNA studies and the

[1]Present address: Department of Biology, University of San Diego, Alcala Park, San Diego, CA 92110 USA.

TABLE 1. Fragment sizes produced by restriction endonuclease digestion of mtDNA purified from albacore tuna from Capetown, South Africa and San Diego, United States. Not all of the smaller fragments were scored with the *Hinf* I and *Msp* I digestions.

Restriction endonuclease	Pattern	Fragment sizes	Sample pattern San Diego	Size and distribution Capetown
Ava I	A	7.6, 4.7, 4.4	10	6
Bam HI	A	14.0, 2.8	8	7
	B	7.0, 7.0, 2.8	—	1
Bst EII	A	12.4, 4.4	4	4
Eco RI	A	8.8, 8.0	8	9
Hinc II	A	3.5, 3.1, 2.9, 2.7, 1.8, 1.6, 1.2	10	9
Hind III	A	4.9, 3.8, 3.0, 1.6, 1.3, 1.2, 0.9	8	9
	B	8.7, 3.0, 1.6, 1.3, 1.2, 0.9	1	—
	C	4.4, 3.8, 3.0, 1.6, 1.3, 1.2, 0.9, 0.5	1	—
Hinf I	A	4.8, 1.2, 1.1, 0.83, 0.71, 0.64, 0.60, 0.51, 0.48, 0.42	3	3
Msp I	A	3.5, 1.4, 1.3, 1.2, 1.1, 0.95, 0.72, 0.56, 0.40	3	3
Pst I	A	10.4, 3.7, 2.7	10	8
	B	14.1, 2.7	1	—
Pyu II	A	8.4, 4.0, 2.7, 1.7	8	6
Sin I	A	8.0, 4.8, 1.3, 1.1, 0.8, 0.7	8	8
	B	7.0, 4.8, 1.3, 1.1, 1.0, 0.8, 0.7	1	—
Xba I	A	9.2, 4.6, 3.0	6	6
Xho I	A	12.0, 4.8	6	6

implications of this investigation are discussed in reference to management of the albacore fishery.

Materials and Methods

Albacore were collected by hook and line (both by trolling artificial lures and live bait fishing) off San Diego, California in July and August 1984 ($n = 11$) and off Capetown, South Africa, in January 1983 ($n = 6$) and February 1985 ($n = 6$). Shortly after capture, liver and gonads were removed from the fish, rinsed in cold buffer (10 mM TRIS pH 7.5, 50 mM EDTA, 250 mM KCl) and quickly frozen on dry ice or in liquid nitrogen. Tissue samples were shipped to the laboratory in liquid nitrogen or on dry ice. Specimens were stored at −75°C until processing.

Preliminary mtDNA analyses were carried out on another scombrid, the chub mackerel, *Scomber japonicus*, to determine optimal tissues for preparing mtDNA as well as to optimize procedures for sample rinsing, freezing, and storage. Highest mtDNA yields in *S. japonicus* were obtained from fresh mature ovaries, although substantial yields were also obtained from fresh liver tissue. Yields of mtDNA decreased with tissue freezing, but were still adequate from liver and ovarian tissues immediately frozen in liquid nitrogen or on dry ice. Rinsing tissues in the cold buffer described above increased yields of closed-circular mtDNA.

We purified mtDNA using CsCl density gradient centrifugation following the protocols presented in Lansman et al. (1981). Restriction endonuclease digestion of purified albacore mtDNA was performed with the following enzymes: *Ava* I, *Bam* HI, *Bst* EII, *Eco* RI, *Hinc* II, *Hind* III, *Hinf* I, *Kpn* I, *Msp* I, *Pst* I, *Pvu* II, *Sin* I, *Xba* I, and *Xho* I. All restriction enzymes were purchased from Bethesda Research Laboratories (BRL), except *Sin* I which was purchased from Sigma Chemical Company, and were used according to the manufacturer's directions. Because of small mtDNA yields from some samples it was not possible to survey each sample with every restriction endonuclease. Sample sizes for each enzyme are presented in Table 1.

Endlabelling of the restriction fragments with radioactive nucleotide triphosphates followed the protocols of Brown (1980) with the exception that we did not find it necessary to ethanol precipitate endlabelled mtDNA fragments resulting from digestion with restriction endonucleases that recognize six nucleotide base pairs. Restriction fragments were separated by size on 1.0% agarose horizontal gels and 3.5–5.0% polyacrylamide vertical gels run at 3 $V \cdot cm^{-1}$ overnight. Gels were dried after electrophoresis, covered with plastic wrap and autoradiographed for 2–24 h without screens at room temperature. Restriction fragment sizes were determined by comparison with commercial size standards (BRL 1 Kbp and 123 bp ladders) run in duplicate or triplicate on each gel.

Results

Results of the restriction endonuclease analysis of albacore mtDNA are summarized in Table 1. Of the 13 restriction endonucleases used in this study, 12 were informative in the sense that they cleaved the circular mtDNA molecule in more than a single site, producing at least two fragments. Restriction fragment length polymorphisms were detected with digestions of *Bam* HI, *Hind* III (2 variants), *Pst* I, and *Sin* I. In all cases each variant was found in a single individual and represented the gain or loss of a single cleavage site from the common pattern. No restriction fragment length polymorphisms were found within a pooled sample of six albacore using *Hinf* I or *Msp* I, restriction enzymes which recognize four nucleotide base pairs. The mtDNA from only three individuals from each ocean was suitable for analysis with these enzymes, which produced several small fragments, because high background counts obscured the smaller fragments in the other individuals.

The size of the albacore mitochondrial genome was estimated by summing the fragment lengths produced by those restriction enzymes that resulted in DNA fragments in the linear (sensitive) region of the size standard. Analysis of fragment sizes resulting from digestions with *Ava* I, *Eco* RI, *Hinc* II, and *Hind* III produced a mean genome size of 16 750 ± 200 nucleotide base pairs for the albacore mitochondrial genome (Table 1). This value lies within the 16 300 to 19 800 base pair range reported for other fish mtDNAs (Avise et al. 1984; Berg and Ferris 1984; Graves et al. 1984; Beckwitt and Petruska 1985; Bentzen et al. 1988).

Discussion

No significant mtDNA sequence differentiation was demonstrated between albacore caught off San Diego, California

and those caught off Capetown, South Africa. There were no restriction fragments which could be used to distinguish north Pacific from south Atlantic albacore. Six restriction fragment length polymorphisms were detected within the pooled sample of 23 individuals. Each of these variants was present in only one individual, and no individual from either ocean was variant for more than a single site.

On the basis of the results obtained in this study, it is not possible to disprove the null hypothesis that albacore from the north Pacific and south Atlantic share a common gene pool. This statement does not preclude the possibility that some genetic differentiation exists between fish from the two ocean basins — a larger investigation, surveying more individuals or enzymes per individual, might demonstrate some mtDNA differentiation. However, with the sample sizes used in this study, both the number of individuals and restriction fragments surveyed, no interocean mtDNA sequence differentiation was detected.

To obtain a relative estimate of the degree of intraspecific differentiation within the albacore population, the results of this study can be compared with the results from other investigations of mtDNA differentiation within vertebrates. At a level of analysis comparable with the present study, both in numbers of individuals and restriction fragments surveyed, significant intraspecific differentiation has been demonstrated in pocket gophers of different counties (Avise et al. 1979), deer mice of different counties (Lansman et al. 1983), and different coastal islands (Ashley and Wills 1987), cotton rats of the same field (Kessler and Avise 1985), bluegill within the same drainage system (Avise and Saunders 1984; Avise et al. 1984), cutthroat trout from different drainage systems (Gyllensten et al. 1985), horseshoe crabs of the Atlantic and Gulf coasts of Florida (Saunders et al. 1986), and toadfishes of the Atlantic coast of the United States (Avise et al. 1987). No such differentiation was found between albacore of the north Pacific and south Atlantic.

The lack of significant mtDNA sequence differentiation between north Pacific and south Atlantic albacore is similar to results reported for the American eel (*Anguilla rostrata*) along the Atlantic coast of North America (Avise et al. 1986) and for skipjack tuna (*Katsuwonus pelamis*) sampled in the Pacific and Atlantic Oceans (Graves et al. 1984). In the case of the American eel, the lack of differentiation probably results from the unusual spawning migration of the fish which may form a single panmictic population (Avise et al. 1986), while the skipjack tuna mtDNA similarity may result from migration between ocean basins (Graves et al. 1984).

While only a few studies have investigated intraspecific mtDNA sequence variation within marine fishes, low levels of intraspecific genetic differentiation of nuclear gene products have been demonstrated for several marine species. In a protein electrophoretic investigation of the milkfish (*Chanos chanos*) Winans (1980) found no significant genetic differentiation between fish sampled over a range of 10 000 km. Shaklee (1984) reports low levels of protein variation within the damselfish (*Stegastes fasciolatus*) sampled throughout the Hawaiian Archipelago, a distance of about 2500 km. Rosenblatt and Waples (1986) found little genetic differentiation between dolphin fish (*Coryphaena hippurus*) of the Pacific and Atlantic or within several species of reef fishes with distributions across the tropical Pacific.

The low level of intraspecific nuclear and mtDNA genetic differentiation observed in these marine fishes may be the result of several factors, but high dispersal ability and a lack of geographic isolating mechanisms in the pelagic environment are probably the most important. Many marine fishes have long-lived pelagic eggs and larvae which are capable of long-range dispersal, and for several of the strong swimming pelagic fishes, such as the tunas and dolphin fish, the ability for long-range dispersal continues throughout the adult life. The great dispersal abilities of these fishes in the pelagic environment, which is essentially free of geographic isolating mechanisms, would tend to result in an increase in intraspecific genetic homogeneity.

The lack of significant intraspecific mtDNA sequence differentiation between north Pacific and south Atlantic albacore encountered in the present study indicates that the two groups have either been separated for a very short time or maintain some form of genetic contact. If gene flow is occurring between albacore of the different ocean basins, then a suitable habitat and mechanism for exchange must exist. Although juveniles of northern and southern oceans are found in northern and southern temperate feeding grounds respectively, adults move toward the tropics to spawn. Tropical spawning seasons are temporally displaced in the northern and southern hemispheres (Sund et al. 1981) and it is not known if there is leakage of adults between spawning areas. Catch rates of albacore are significantly depressed near the equator; however, positive catches during different seasons across the equator in the western Pacific indicate that the distribution of albacore may at times be continuous (Foreman 1980). In the southern oceans albacore are taken as far south as 45°S in waters above the west wind drift. As albacore from all three southern oceans may be feeding in these waters, there is ample opportunity for exchange between southern ocean basins via the Indian Ocean.

The number of albacore needed to migrate between ocean basins to prevent genetic differentiation from accumulating is very small. Theoretical models of nuclear gene product differentiation (Allendorf and Phelps 1981) or mtDNA differentiation (Takahata and Palumbi 1985) indicate that migration on the order of individuals per generation is sufficient to prevent genetic differentiation. This gene flow does not have to be constant and does not have to represent the long migration of any individual fish; rather, it can be periodic and occur as several short exchanges. Inasmuch as albacore in all oceans move to the tropics to spawn, the possibility of such exchanges in matings between fish from different areas seems likely.

This study was undertaken to determine the magnitude of mtDNA sequence differentiation between north Pacific and south Atlantic albacore so that we could estimate the sampling effort required to separate possible stocks within the north Pacific. If the variant mtDNA restriction patterns found in single albacore of one ocean in this study represent real differences in the presence and absence of mtDNA clones between albacore of the north Pacific and south Atlantic (and are not artifacts of sampling error), a considerably larger sample size from each ocean would be required to statistically distinguish the populations. Such an investigation is certainly feasible; however, assuming that intraocean genetic differentiation is considerably less than interocean differentiation, a sampling effort of tremendous magnitude would be required to determine if genetic differentiation exists between putative albacore stocks within the north Pacific. Rather than commit resources to such a study at this time, we believe that a reevaluation of the problem is warranted.

The results of this analysis indicate that there is a high degree of mtDNA sequence similarity between albacore of the north Pacific and south Atlantic. Although albacore of the north Pacific and south Atlantic may represent a single, homogeneous stock to the population biologist, they do not represent a single management unit to the fishery biologist. While the level of gene flow between ocean basins may be sufficient to prevent significant genetic differentiation from accumulating, it is doubtful if the gene flow represents even a minor fraction of the total population. On a smaller scale, the two putative populations of albacore which reach the west coast of North America may have as much as 4% interannual leakage (Laurs and Nishimoto 1979). While 4% interannual exchange between fish returning north or south of 40°N represents a significant gene flow on an evolutionary time scale to the population biologist, it also represents a significant stock boundary to the fishery biologist. The problem may be semantic, but it is indicative of a current misunderstanding of the role of genetic analyses in fishery management. If genetic differentiation can be demonstrated between two fishery units separated on the basis of fishery statistics or geographic location, they are distinct stocks. However, if no genetic differentiation can be demonstrated between these units, they may still be managed as putative stocks, even though they are genetically homogeneous.

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