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Global population structure of albacore (*Thunnus alalunga*) inferred by RFLP analysis of the mitochondrial ATPase gene

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Abstract The genetic population structure of the highly migratory albacore (*Thunnus alalunga*) was investigated using restriction fragment length polymorphism (RFLP) analysis of the mitochondrial ATPase gene amplified by the polymerase chain reaction (PCR). 620 individuals comprising 13 geographically distant samples (ten Pacific, two Atlantic and the Cape of Good Hope) were surveyed between 1991 and 1994 with two restriction endonucleases (*Mse* I and *Rsa* I), resulting in seven haplotypes. No heterogeneity was observed in the distribution of haplotypes among the ten samples from the North and South Pacific, nor among the samples from North and South Atlantic and Cape of Good Hope. However, highly significant heterogeneity was evident among Atlantic and Pacific samples. Higher haplotypic diversity (*h*) was observed in the Pacific samples (0.59 to 0.69) than in the Atlantic and Cape samples (0.22 to 0.43). These results suggest greater gene flow between albacore of the northern and southern hemispheres (within oceans) than between the Atlantic and Pacific Oceans.

Introduction

Albacore (*Thunnus alalunga*) is a highly migratory cosmopolitan fish, found in tropical, sub-tropical and temperate areas of all oceans from about 45°N to 50°S, including the Mediterranean Sea (Collette and Nauen 1983). Albacore populations of each ocean basin have been considered as different stocks as well as the populations of the northern and southern hemispheres. Morphometric differences have been reported between the specimens of different oceans or hemispheres or even between western and eastern basins within an ocean (Godsill 1948; Kurogane and Hiyama 1958, 1959; Ishii 1965). Catch statistics, length data and tagging have also been used for stock analysis (Otsu 1960; Clemens 1961; Otsu and Uchida 1962; Koto 1968; Morita 1977).

Lewis (1990) suggested that stock analysis may be attempted using more simple heritable characters. Suzuki (1962) first applied biochemical analysis for studying albacore stock structure and reported significant differences in the frequencies of antigens between oceanic specimens. But the genetic basis of this "blood type" was not clarified, and the antibodies used are no longer available. Restriction fragment length polymorphism (RFLP) analysis on mitochondrial DNA (mtDNA) has become the preferred method for investigating genetic variation within fish species. Graves and Dizon (1989) using this method found no characteristic restriction sites to descriminate small samples of albacore from the South Atlantic and North Pacific. Their results suggested that genetic stock studies of this species must be conducted using individual haplotype analysis based on a larger sample size. Chow et al. (1993) demonstrated that considerable levels of genetic polymorphism may be detected by means of restriction analysis even in the short DNA fragment amplified by polymerase chain reaction (PCR), and Chow and Inoue (1993) applied this method for detecting intra- and inter-specific genetic polymorphisms in tuna species, observing relatively high polymorphism in the mitochondrial ATPase gene fragment of the albacore.

PCR–RFLP analysis can be performed on ethanol-preserved tissues, facilitating sampling and transportation of the specimens. Indeed, upon our request several research organizations quickly responded and sent us small pieces of muscle tissue preserved in ethanol from many individuals. The PCR–RFLP technique is simple and relatively inexpensive, making it well suited for the analysis of a large sample size. In the present study we employed PCR–RFLP analysis of the albacore

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mitochondrial ATPase gene to infer the global stock structure of the species.

Materials and methods

Collection information is presented in Fig. 1 and Table 1, including sample locations, dates, mean fork length of the albacore samples used in this study, and the organizations that collaborated on sample collection. A small piece of muscle tissue (ca. 0.1 to 5 g) was dissected from fresh or frozen fish on board or at the landing site and placed in a plastic bag or a 1.5 ml micro-centrifuge tube. All samples collected by the Bureau of Resource Science (BRS), Fisheries Institute of Sao Paulo (IP-SP), Institute Francais de Recherche pour L'exploitation de la Mer (IFREMER), National Marine Fisheries Service (NMFS) and South Pacific Commission (SPC) were fixed in ethanol and air-mailed to the laboratory in Japan. Those specimens collected by the National Research Institute of Far Seas Fisheries (NRIFSF) and Japan Marine Fishery Resources Research Center (JAMARC) were transferred to the laboratory of the NRIFSF fresh or frozen. The procedures for DNA extraction and amplification of the mitochondrial ATPase gene are described in Chow et al. (1993) and Chow and Inoue (1993), with the exception that the PCR amplification was carried out in 12 µl reaction mixture in the present study. The nucleotide sequences of the primers used were (L8562) 5′-CTTCGACCAATTTATGAGCCC-3′ and (H9432) 5′-GCCATATCGTAGCCCTTTTTG-3′. Two endonucleases (*Mse* I and *Rsa* I) out of 20 tested were reported to detect polymorphisms in this species (Chow and Inoue 1993). The amplified DNAs were directly digested by each of the endonucleases and electrophoresed through 2.5 to 3% agarose gel (Biogel, BIO101 Co.). Nucleon diversity (h) (Nei and Tajima 1981) of each local sample was

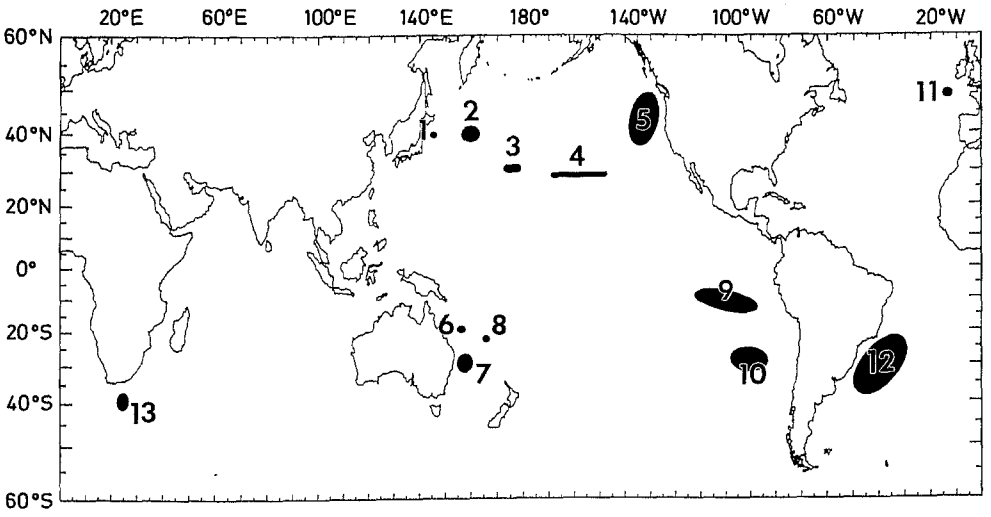

Fig. 1 *Thunnus alalunga.* Map showing catch localities of albacore samples used in study. See Table 1 for detailed information

Table 1 *Thunnus alalunga.* Catch locality, date and mean fork length of the albacore samples used in study. *BRS* Bureau of Resource Sciences (Australia), *IP-SP* Fisheries Institute of Sao Paulo (Brazil); *IFREMER* Institute Francais de Reacherche pour L'exploitation de la Mer (France); *JAMARC* Japan Marine Fishery Resources Research Center (Japan); *NMFS* National Marine Fisheries Service (USA); *NRIFSF* National Research Institute of Far Seas Fisheries (Japan); *SPC* South Pacific Commission (New Caledonia)

Area Sample	Latitude	Longitude	Date	Collaborating organization	Position in Fig. 1	Mean fork length ± SD
North Pacific						
NW Pacific 1	38N	144E	1 Oct 1991	NRIFSF	1	55.0 ± 1.3
NW Pacific 2	36–40N	159–165E	23 Jun–19 Sep 1993	NRIFSF	2	62.1 ± 14.1
Empelor Sea Mounts	32–33N	173–179E	16 May 1993	JAMARC	3	72.5 ± 7.8
Hawaii	30N	145–160W	4–22 Apr 1992	JAMARC	4	69.7 ± 5.4
NE Pacific	34–51N	126–140W	28 Jun–31 Aug 1993	NMFS	5	64.8 ± 2.5
South Pacific						
E Australia 1	19S	153–154E	1–4 Aug 1993	BRS	6	91.9 ± 5.1
E Australia 2	29–33S	153–159E	30 Jul–14 Aug 1993	BRS	7	83.5 ± 10.0
New Caledonia	22S	166E	7–14 Dec 1993	SPC	8	99.6 ± 4.0
Off Peru	9–18S	84–116W	11 Jun 1993–11 Jan 1994	JAMARC	9	95.7 ± 7.0
Off Chile	28–34S	85–99W	16 Aug–23 Nov 1993	JAMARC	10	84.8 ± 10.7
North Atlantic						
NE Atlantic	48N	13W	16 Sep 1993	IFREMER	11	62.6 ± 3.3
South Atlantic						
Off Brazil	25–35S	35–50W	28 Feb–18 Mar 1994	IP-SP	12	101.5 ± 4.4
Indo Atlantic						
Cape of Good Hope	38–42S	24–26E	29 Apr–20 Jun 1992	NRIFSF	13	78.9 ± 18.2

estimated using frequencies of the haplotypes detected. The *G*-test of independence (Sokal and Rohlf 1981) was used to determine whether the haplotype proportions were independent of the localities.

Results

RFLP

Mse I and *Rsa* I digestions generated five and three restriction patterns, respectively, which were labelled alphabetically (Fig. 2). The number and frequency of these restriction patterns in each sample are shown in Table 2. For both endonucleases, two restriction patterns (designated *A* and *B*) were common in all samples. The *C*, *D* and *E* patterns of *Mse* I were very rare; the *C* pattern was observed only for one individual each in the NW Pacific 2 and Chile samples. Two individuals of the *D* pattern were found in the Empelor Sea Mounts sample and one in the Hawaii. One individual having the *E* pattern was found in Peru sample. The *C* pattern in *Rsa* I digestion was also very rare and was found only in one individual of the NW Pacific 1 sample.

Based on the frequency of the restriction patterns, the samples can likely be categorized into two large groups. Frequencies of the *A* pattern in *Mse* I digestions ranged from 61 to 83% in the Pacific samples and from 82.7 to 91.2% in the other samples. For *Rsa* I digestions, the frequency of the *A* pattern ranged from 71 to 87.8% in the Pacific samples and from 90.7 to 97.1% in the other samples.

Haplotype analysis

The frequency of composite haplotypes among samples was compared, as haplotype analysis is more sensitive than simply comparing frequencies of individual restriction patterns for detecting heterogeneity between samples. Among 620 individuals analyzed on the 13 local samples, seven haplotypes were detected. The number and frequency of the haplotypes and *h* for each sample are presented in Table 3, and a pie graph of haplotype frequencies is presented in Fig. 3. Haplotype *AA* was most commonly observed in all samples followed by *BA* and *AB*. The other four haplotypes were found to be very rare. Striking differences in the haplotype frequencies were observed between samples. The frequency of *AA* type ranged from 41 to 57.6% in the Pacific samples, while those ranged from 73.4 to 88.2% in the Atlantic. Likewise, the frequencies of *BA* and *AB* types were lower in the Atlantic collections than in the Pacific. Haplotypic diversities were found to be much higher in the Pacific samples (59 to 69%) than in the Atlantic and Cape collections (22 to 42.8%).

The number of those three haplotypes (*AA*, *BA* and *AB*) were used to test heterogeneity between the specimens. The results of pairwise comparison between the

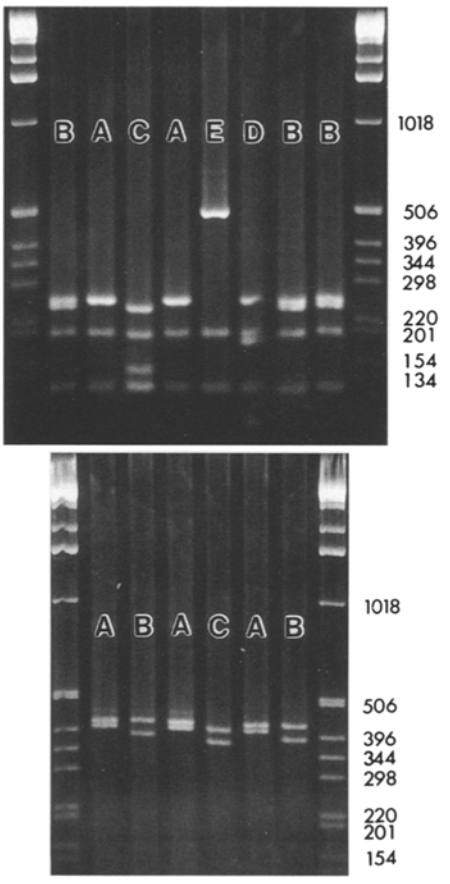

Fig. 2 *Thunnus alalunga.* Gel electrophoresis of mitochondrial ATPase gene fragment digested by *Mse* I (top) and *Rsa* I (bottom), showing restriction fragment length polymorphisms. Left and right ends are 1 kb DNA ladder (GIBCO, BRL) and sizes are indicated along right margin (*bp* base pairs). Estimated fragment sizes (bp) of each restriction pattern in *Mse* I digestion are: *A*: 260, 195, 115; *B*: 260, 250, 195, 115; *C*: 250, 195, 145, 115; *D*: 260, 195, 180, 115, 80; *E*: 500, 195, 115. Those in *Rsa* I are: *A*: 420, 405; *B*: 420, 380; *C*: 405, 370

samples using the *G*-test are shown in Table 4. No heterogeneity was evident among the Pacific samples. No heterogeneity was observed among samples of the Atlantic and Cape of Good Hope. In addition, no significant difference was observed between size classes within a sample and between samples of different years within each ocean (results not shown). In contrast, highly significant heterogeneity was evident between the Pacific and Atlantic/Cape of Good Hope samples except between the Cape sample and three Pacific samples (NW Pacific 2, New Caledonia and Chile).

Discussion

Haplotype analysis of the albacore mitochondrial ATPase gene revealed genetic heterogeneity between the Pacific and Atlantic albacore samples, but suggested homogeneity within each ocean. Based on tag

Table 2 *Thunnus alalunga.* Number and % frequency (parentheses) of restriction patterns (*A*, *B*, *C*, *D*, *E*) of mitochondrial ATPase gene fragment digested by *Mse* I and *Rsa* I

Sample	*Mse* I						*Rsa* I			
	A	*B*	*C*	*D*	*E*	Total	*A*	*B*	*C*	Total
NW Pacific 1	25 (61.0)	15 (36.6)	1 (2.4)	0	0	41	34 (82.9)	7 (17.1)	0	41
NW Pacific 2	43 (76.8)	13 (23.2)	0	0	0	56	43 (76.8)	12 (21.4)	1 (1.8)	56
Empelor Sea Mounts	33 (70.2)	12 (25.5)	0	2 (4.3)	0	47	34 (72.3)	13 (27.7)	0	47
Hawaii	38 (71.7)	14 (26.4)	0	1 (1.9)	0	53	40 (75.5)	13 (24.5)	0	53
NE Pacific	39 (76.5)	12 (23.5)	0	0	0	51	36 (73.5)	13 (26.5)	0	49
E Australia 1	26 (83.9)	5 (16.1)	0	0	0	31	22 (71)	9 (29)	0	31
E Australia 2	24 (70.6)	10 (29.4)	0	0	0	34	26 (76.5)	8 (23.5)	0	34
New Caledonia	41 (78.8)	11 (21.2)	0	0	0	52	41 (78.8)	11 (21.2)	0	52
Off Peru	26 (66.6)	12 (30.8)	0	0	1 (2.6)	39	28 (71.8)	11 (28.2)	0	39
Off Chile	32 (65.3)	16 (32.7)	1 (2)	0	0	49	43 (87.8)	6 (12.2)	0	49
NE Atlantic	31 (91.2)	3 (8.8)	0	0	0	34	33 (97.1)	1 (2.9)	0	34
Off Brazil	52 (86.7)	8 (13.3)	0	0	0	60	54 (93.1)	4 (6.9)	0	58
Cape of Good Hope	62 (82.7)	13 (17.3)	0	0	0	75	68 (90.7)	7 (9.3)	0	75

Table 3 *Thunnus alalunga.* Number and % frequency (parentheses) of haplotypes

Sample	*Mse* I/*Rsa* I					Haplotypic diversity (%)
	A	*BA*	*AB*	Others	Total	
NW Pacific 1	18 (43.9)	15 (36.6)	7 (17.1)	1 (2.4)	41	66.0
NW Pacific 2	30 (53.6)	13 (23.2)	12 (21.4)	1 (1.8)	56	62.4
Empelor Sea Mounts	20 (42.6)	13 (27.7)	12 (25.5)	2 (4.2)	47	69.0
Hawaii	25 (47.2)	14 (26.4)	13 (25.5)	1 (1.9)	53	65.4
NE Pacific	23 (48.9)	11 (23.4)	13 (27.7)	0 (1.8)	51	64.2
E Australia 1	17 (54.9)	5 (16.1)	9 (29)	0	31	60.9
New Caledonia	30 (57.6)	11 (21.2)	11 (21.2)	0	52	59.0
Off Peru	16 (41)	12 (30.7)	10 (25.7)	1 (2.6)	39	68.9
Off Chile	26 (53.1)	16 (32.7)	6 (12.2)	1 (2)	49	60.8
NE Atlantic	30 (88.2)	3 (8.8)	1 (3)	0	34	22.0
Off Brazil	46 (79.3)	8 (13.8)	4 (6.9)	0	58	35.3
Cape of Good Hope	55 (73.4)	13 (17.3)	7 (9.3)	0	75	42.8

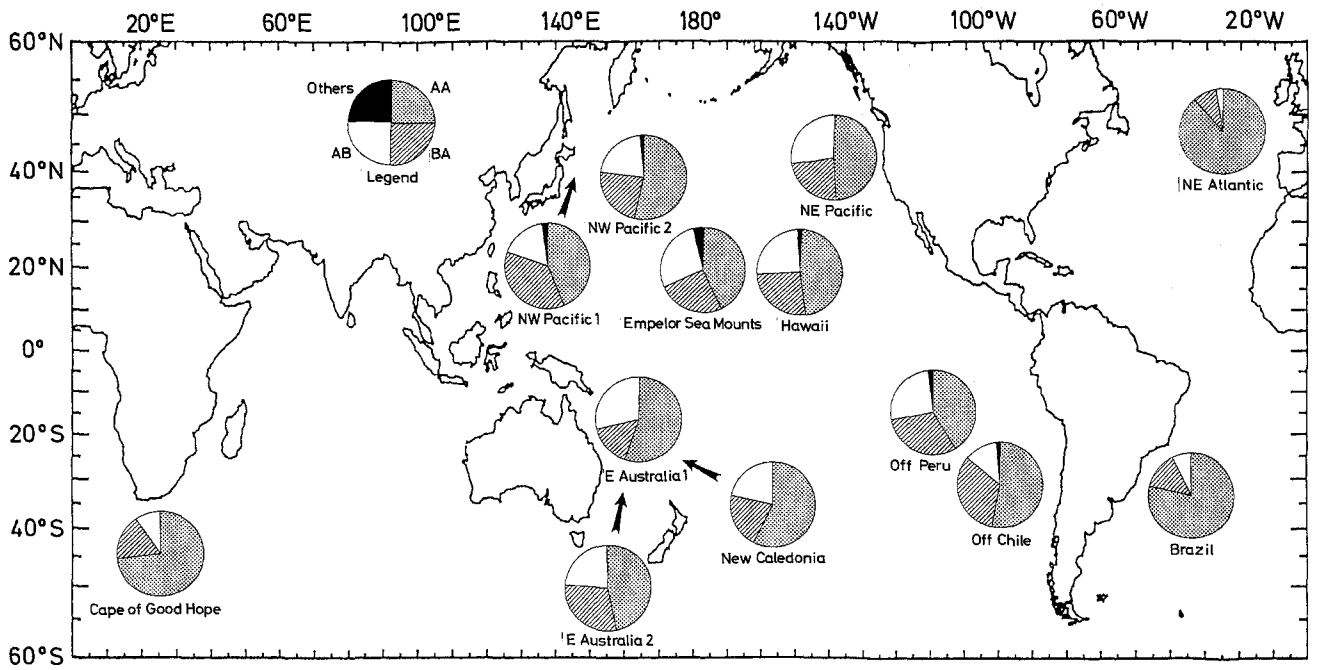

Fig. 3 *Thunnus alalunga.* Pie graph representation for the haplotype frequencies of 13 local albacore samples

recovery data, age and growth information, and distribution and size frequency data, Otsu (1960), Clemens (1961), and Otsu and Uchida (1962) suggested that there is a single albacore stock in the North Pacific. Though Godsil (1948) reported morphometric differences between western and eastern Pacific albacore samples, the apparent panmixia within the North Pacific albacore population is consistent with the results of the present study.

In contrast, isolation between the albacore populations of the North and South Pacific has been considered to be much stronger. Nakamura (1969) pointed out several differences in fishing grounds and seasonal and local variations in hook rates observed between the North and South Pacific. He also noted that in the North Pacific the habitats of the feeding and spawning groups are clearly separated by the Subtropical Convergence, while such a division is lacking in South Pacific albacore. Notable differences in morphometry were also reported by Kurogane and Hiyama (1958, 1959) and Ishii (1965). Thus, migration across the equator between these stocks is assumed to be negligible (Nakamura 1969; Lewis 1990). However, the haplotype frequency and haplotypic diversities obtained in the present study were remarkably stable throughout the wide range of the Pacific Ocean. Supplemental analysis of these values between size or year classes also support the homogeneous feature of the Pacific albacore population. Similar results were obtained by Scoles and Graves (1993), who performed restriction analysis on mtDNA of yellowfin tuna (*Thunnus albacares*) and suggested that there is sufficient gene flow within the Pacific. Hook rates of albacore in the equatorial region (between 10 °N and 5 °S) of the western Pacific was much lower than in the other areas, i.e., north of 10 °N and south of 5 °S, but are not zero (Suzuki et al 1977). Histological observations of albacore gonads indicated that central equatorial Pacific fish exhibited early to late stages of ovary development (Otsu and Uchida 1959), strongly suggesting the occurrence of spawning in the equatorial area. Thus, we propose that there could be gene flow between the North and South Pacific albacore populations which prevents the accumulation of genetic differentiation. This suggestion is also applicable to the relationships between the North and South Atlantic albacore populations and is supported by catch statistics from long-line fishing, which Koto (1968) used to suggest intermingling of mature albacore from the North and South Atlantic.

The apparent genetic heterogeneity between the Atlantic and Pacific albacore populations presented in our study coincides with Suzuki (1962), who reported a highly significant difference in the antigen frequencies between the Atlantic and Pacific albacore samples. One may pose the question whether or not a boundary between ocean populations occurs and, if so, where, or if the Atlantic and Pacific albacore samples represent extremes of one large population in which gene flow is somehow restricted by "isolation by distance". Suzuki (1962) also analyzed albacore samples from the Indian Ocean and reported that the antigen frequencies of Indian Ocean albacore were significantly different from those of the Atlantic and Pacific, but were most similar to those of the Pacific. Although the haplotype frequencies of the Cape of Good Hope sample in the present study were not significantly different from those of the

Table 4 *Thunnus alalunga*. Pairwise comparison on the haplotype frequencies between albacore samples using *G*-statistics

No.	Sample location	Sample											
		1	2	3	4	5	6	7	8	9	10	11	12
1	NW Pacific 1												
2	NW Pacific 2	2.13											
3	Empelor Sea Mounts	1.29	1.01										
4	Hawaii	1.44	0.45	0.13									
5	NE Pacific	2.49	0.51	0.37	0.19								
6	E Australia 1	4.37	0.97	1.76	1.33	0.64							
7	E Australia 2	0.90	0.52	0.11	0.07	0.42	1.65						
8	New Caledonia	2.98	0.13	1.72	0.98	0.84	0.78	1.06					
9	Off Peru	0.93	1.43	0.08	0.35	0.74	2.34	0.18	2.23				
10	Off Chile	0.84	2.16	3.04	2.63	3.75	4.87	1.69	2.55	2.82			
11	NE Atlantic	16.34*	12.71*	18.19*	16.59*	15.89*	11.37*	14.50*	10.82*	18.44*	11.61*		
12	Off Brazil	12.34*	8.70*	14.27*	12.65*	12.15*	8.21*	10.39*	6.94*	14.41*	7.81*	1.35	
13	Cape of Good Hope	8.97*	5.70	10.74*	9.21*	9.03*	6.21*	7.31*	4.34	10.89*	5.03	3.45	0.65

* Significantly different ($df = 2$). Critical values are $P_{0.05} = 5.991$, $P_{0.01} = 9.21$ and $P_{0.005} = 10.597$

North and South Atlantic samples, the lower frequency of the *AA* haplotype and higher *h* than the Atlantic samples may suggest affinity of this sample to the Pacific group. Indeed, the haplotype frequencies of the Cape sample were not significantly different from those of some Pacific samples. Based on the length frequency distributions of spawning albacore between the South Atlantic and Indian Oceans and the presumed migratory movement of fish, Koto (1968) suggested that intermingling of immature fish may occur between the southern Atlantic and southern Indian Oceans, but this does not apply for mature fish. Morita (1977) assumed that there was migration between the Atlantic and Indian Oceans. Although both Koto (1968) and Morita (1977) admitted that albacores from the Atlantic and Indian Oceans may concurrently occur off South Africa, the former author presumed very little gene flow between the two groups in contrast to the latter author. Our Cape sample might be a mixture, but it is not possible to test heterogeneity within the sample because of the haploid nature of the mtDNA molecule. An analysis of albacore polymorphic nuclear genes will clarify those relationships.

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