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Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa

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Abstract

Two mitochondrial DNA segments of the bigeye tuna (*Thunnus obesus*) were amplified by polymerase chain reaction (PCR), and restriction fragment length polymorphism (RFLP) analyses of these segments were used for the genetic stock study. The variation in a segment flanking the ATPase and COIII genes was low; only two genotypes (α and β) were detected by *Rsa*I digestion. Yet a large difference in the genotype distribution was observed between ocean basin samples. The α type predominated in four Atlantic samples, where 178 of 244 individuals were the α type. In contrast, only one of 195 individuals collected in the Indo-Pacific was the α type? The frequency of the α type varied considerably from 0 to 80% among seven samples collected off the Cape of Good Hope. The variation found in the other segment, containing the D-loop region, was much higher; two endonucleases (*Dpn*II and *Rsa*I) detected five genotypes each and 15 composite genotypes. A highly significant difference in genotype frequencies was observed between the Atlantic and Indo-Pacific samples, but no heterogeneity was observed among the four Atlantic or among four Indo-Pacific samples. These results clearly indicate that not only gene flow, but also fish migration, between the Atlantic and Indian Oceans are severely restricted, and that fishes from these distinct stocks are intermingling around South Africa. The simple and diagnostic genetic marker found in this study can be used to estimate mixing ratios between Atlantic and Indian stocks around South Africa.

Keywords: bigeye tuna, mtDNA, population mixture, population subdivision

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Introduction

The bigeye tuna (*Thunnus obesus*) is a large epi- and mesopelagic scombrid fish, inhabiting all oceans except the Mediterranean Sea (Collette & Nauen 1983). Warm surface waters are the main habitat for young juveniles, as surface fisheries operating in tropical and subtropical areas catch substantial numbers of young juveniles, occasionally with just a few adults. Adult and subadults have a wider distribution than juveniles as they tolerate an oxygen-depleted habitat and have the ability to penetrate deeper and cooler waters (Hanamoto 1976; 1987; Holland *et al*. 1992; Brill 1994). Thus, bigeye tuna appear to shift or expand their habitat from surface to deeper waters and from tropical to temperate zones as they grow. These biological aspects, and data from fishing areas, size structure and distribution of fish, sexual maturity, tagging, and larval distribution have allowed us to assume that there is a single bigeye stock in each ocean basin (see reviews by Miyabe & Bayliff (1998), Pallares *et al*. (1998) and Stobberup *et al*. (1998)). Another problem deserving attention is the possibility of the mixing of fishes between oceans. Kume *et al*. (1971) mentioned that high hook rates observed off South Africa in all seasons may be indicative of the mixing of fishes from the Indian and Atlantic Oceans.

Understanding fish stock structure may improve fishery management. If multiple stocks occupy the same area, they

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Table 1 Descriptions for the bigeye tuna samples used in this study

Ocean basin area	Sample code	Latitude	Longitude	Date	Fishing gear
Atlantic Ocean					
Northwest	NWA	37–41N	48–67W	April 1997	Longline
Central north	CNA	5–8N	8–21W	January–March 1997	Longline
Central south	CSA	5–11S	2E–8W	January–February 1997	Longline
Southwest	Brazil	20–33S	40–50W	September 1996–May 1997	Longline
South Africa					
East	Cape-1	38–41S	24–25E	May–June 1992	Longline
	Cape-2	na	na	November 1997	Longline
	Cape-3	na	na	December 1997	Longline
	Cape-4	na	na	September 1998	Longline
	Cape-5	na	na	September 1998	Longline
West	Cape-6	40S	15E	August 1998	Longline
East	Cape-7	40S	25E	August 1998	Longline
Indian Ocean					
East	E.Ind	5S–5N	85–90E	May 1996	Purse sein
Pacific Ocean					
West	Celebes	Celebes Sea		April–August 1993	Purse sein
Central	CWP	0–5N	140–145E	May 1994	Purse sein
East	Peru	8–17S	84–124W	June–July 1994	Longline

should be managed separately and we have to determine the mixing ratio of individuals in the catch. In highly migratory tuna and billfish species, biochemical genetic analyses have revealed substantial genetic heterogeneity among ocean basin samples or even among samples within an ocean basin (Fujino 1970; Ward *et al*. 1994; Chow & Ushiama 1995; Graves & McDowell 1995; Kotoulas *et al*. 1995; Rosel & Block 1996; Chow *et al*. 1997; Alvarado-Bremer *et al*. 1998). There have been only two biochemical genetic analyses on bigeye tuna populations. Suzuki (1962) examined the Tg blood type and found no difference between Indian and Pacific samples. Alvarado-Bremer *et al*. (1998) examined the mitochondrial DNA (mtDNA) control region and also found no difference in genotype frequencies between Indian and Pacific samples, but both considerably differed from Atlantic samples, indicating genetic distinctness between the Atlantic and Indo-Pacific stocks. Although these studies were performed to test whether or not the samples analysed were heterogeneous, most of the genetic markers were not straightforward for estimating mixing ratios. When we realize that a fish population is structured, we should investigate diagnostic and practical genetic markers for assessing the extent and ratio of mixing between stocks.

Here we report our finding that the global population of bigeye tuna is subdivided into two distinct stocks (Atlantic and Indo-Pacific) and that fishes from these stocks mix around South Africa with very little gene flow. The genetic marker used in this study is simple and may be used for estimating the mixing ratio between these two stocks.

Materials and methods

The collection information on the 15 samples of bigeye tuna (*Thunnus obesus*) used in this study are presented in Table 1. Four samples from the Atlantic Ocean were collected by on-board observers in the northwest (NWA), central north (CNA), central south (CSA), and southwest (Brazil) Atlantic. Six of seven 'Cape of Good Hope' samples (Cape-1 to 7) were collected at unloading sites in Japan and one was collected by an on-board observer. The collection locality information was available in three (Cape-1, 6 and 7) of the seven Cape samples. One sample from the Indian Ocean (E. Ind) was collected by the RV *Nippon-Maru* cruise in the eastern Indian Ocean. Bigeye tuna from Celebes Sea (Celebes) were all juveniles caught by artisanal fisheries and collected at unloading sites in General Santos City, Philippines. The other two Pacific samples were from the central western Pacific (CWP) and the southeastern Pacific (Peru) and were collected at the landing site in Japan and by an on-board observer, respectively. The procedures for total DNA extraction from frozen or ethanol-preserved muscle tissue and polymerase chain reaction (PCR) amplification followed by restriction fragment length polymorphism (RFLP) analysis are described elsewhere (Chow & Inoue 1993; Chow & Ushiama 1995; Chow *et al*. 1997). Two primer sets to amplify two mtDNA segments, one containing the control region (D-loop) and the other containing the flanking region between the ATPase 6 and cytochrome oxidase subunit III genes (*ATCO*), were from Palumbi *et al*. (1991) and Chow & Inoue (1993), respectively. The PCR reaction,

scaled down to a 8 μL total volume, was carried out with an initial denaturation at 95 °C for 1 min followed by 30 cycles of amplification (denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 2 min, with a final extension at 72 °C for 10 min). The same amplification conditions were used for both mtDNA segments. The *ATCO* segment has been used for identifying tuna species of the genus *Thunnus* (Chow & Inoue 1993; Chow & Kishino 1995), with bigeye tunas showing characteristic restriction profiles in *Mse*I and *Rsa*I digestions. A preliminary investigation revealed that the Atlantic samples were polymorphic but Pacific samples were monomorphic in these enzyme digestions, and in the present study *Rsa*I, showing the simplest restriction profile, was applied to all samples. For the D-loop segment, two endonucleases (*Dpn*II and *Rsa*I) revealed relatively high and clear RFLP, and these two enzymes were applied to all samples. The restricted PCR products were electrophoresed in a 2.5% agarose gel (Biogel, BIO101) in 0.5 × TBE buffer (90 mM Tris-boric acid, and 2 mM EDTA). Chi-square analysis was conducted using the MONTE CARLO simulation of Roff & Bentzen (1989) with 1000 randomizations of the data to test the heterogeneity of genotype distributions among samples.

Results

ATCO segment

Only two restriction types (designated genotypes α and β) were detected in the *ATCO* segment by *Rsa*I digestion. A marked difference in genotype distribution was found between the Indian and Atlantic Ocean samples (Fig. 1). Both genotypes were present in the Atlantic sample,

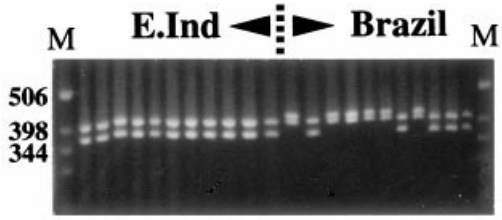

Fig. 1 Restriction profile of the mitochondrial DNA (mtDNA) *ATCO* segment of bigeye tunas digested by *Rsa*I. E.Ind and Brazil are samples from the eastern Indian Ocean and South Atlantic, respectively. Two types (α and β) are observed in the Brazil sample but only one type (β) in the E.Ind sample. M is a 1 kb DNA ladder and sizes are indicated along the left margin.

while only the β type was observed in the Indian sample. The frequencies of these two genotypes in all samples are presented in Table 2. The seven Cape samples were pooled. The α type predominated in the four Atlantic samples, with 178 (73%) of 244 individuals. In contrast, the E.Ind and two Pacific (Celebes and CWP) samples were completely monomorphic for the β type, and no α type individual was observed. Only one (1.8%) of 57 individuals in the southeast Pacific (Peru) sample was α type. The frequency of the α type in the pooled Cape sample was intermediate (19.8%). The distribution of the two genotypes among the nine samples was highly heterogeneous ($P < 0.001$), while no heterogeneity was observed among the four Atlantic samples ($P = 0.144$) or among the four Indo-Pacific samples ($P = 0.703$). Incorporating the Cape sample in the Atlantic or Indo-Pacific samples resulted in significant heterogeneity among samples ($P < 0.001$). The genotype frequencies of the seven Cape samples are shown in Table 3, in which the frequency of

Table 2 Genotype frequencies and genotype diversity (*H*) in mitochondrial DNA (mtDNA) *ATCO* segments of nine bigeye samples

Enzyme genotype	Atlantic				Cape total	Indian E.Ind	Pacific		
	NWA	CNA	CSA	Brazil			Celebes	CWP	Peru
RsaI									
α	51	49	50	28	20	0	0	0	1
β	29	14	15	8	72	51	43	44	56
H	0.468	0.351	0.361	0.356	0.344	0	0	0	0.035
n	80	63	65	36	92	51	43	44	57

Table 3 Genotype frequencies and genotype diversity (*H*) in mitochondrial DNA (mtDNA) *ATCO* segments of seven bigeye samples collected off the Cape of Good Hope

	Cape-1 24–25E	Cape-2	Cape-3	Cape-4	Cape-5	Cape-6 15E	Cape-7 25E
α	2	3	8	1	2	3	1
β	10	2	2	9	5	16	28
H	0.303	0.600	0.356	0.200	0.476	0.281	0.069
n	12	5	10	10	7	19	29

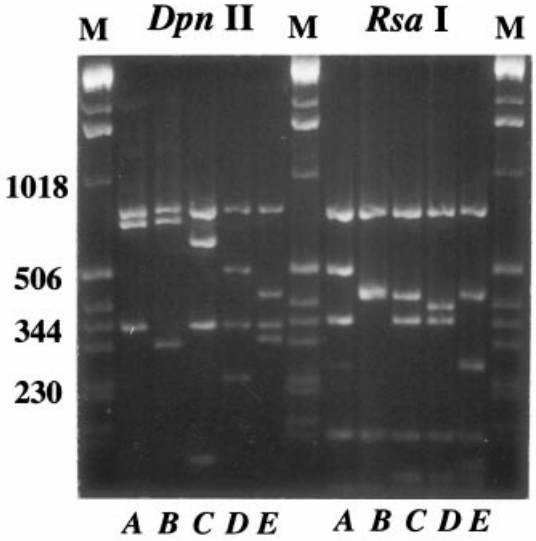

Fig. 2 Restriction profiles of the mitochondrial DNA (mtDNA) D-loop segment of bigeye tunas digested by *Dpn*II and *Rsa*I. The five genotypes observed in each digestion are labelled alphabetically (*A*–*E*). M is a 1 kb DNA ladder and sizes are indicated along the left margin.

the α type varied considerably from sample to sample, ranging from 0 to 80%. There was highly significant heterogeneity among the Cape samples ($P < 0.001$).

D-loop segment

Restriction profiles in the D-loop segment obtained by *Dpn*II and *Rsa*I digestions are shown in Fig. 2. Each enzyme revealed five genotypes (designated *A* to *E*). Compositing these restriction types yielded 15 composite genotypes among the 477 individuals examined (Table 4). The most common genotype in the Atlantic samples was *BB*, which occurred at a frequency from 43.8 to 63.2% of individuals. In contrast, this genotype was nearly absent in the Indo-Pacific samples (only one in the Peru sample). The genotype distribution over all nine samples was highly heterogeneous ($P < 0.001$). In contrast, no heterogeneity was observed among the four Atlantic samples ($P = 0.432$) nor among the four Indo-Pacific samples ($P = 0.382$). Incorporation of the Cape sample into the Atlantic samples resulted in high heterogeneity ($P < 0.001$), while incorporation into the Indo-Pacific samples gave marginal heterogeneity ($P = 0.014$). Composite genotypes in the D-loop segment were classified by the α and β genotype frequencies and genotype frequencies were compared among samples. All β type individuals were pooled in each of three putative classes (Atlantic, Cape and Indo-Pacific) to compare the D-loop genotype distributions (Table 4). There was no heterogeneity in the D-loop genotype distributions of the β type among the Atlantic, Cape and Indo-Pacific samples ($P = 0.072$).

Discussion

Large differences in genotype distributions between bigeye tuna samples from the Atlantic and Indo-Pacific were first found by Alvarado-Bremer *et al*. (1998). Using nucleotide sequencing analysis of a short segment of mtDNA control region, they found that mtDNA genotypes

Table 4 Composite genotype frequencies and genotype diversity (*H*) in mitochondrial DNA (mtDNA) D-loop segments of nine bigeye tuna samples and those of β type individuals

	Atlantic				Cape	Indian	Pacific					
	NWA	CA	SA	Brazil	total	E.Ind	Celebes	CWP	SEP	Atlantic β	Cape β	Indo-Pacific β
AA	0	0	1	0	0	2	2	3	3	1	0	10
AB	3	2	0	1	3	3	3	6	3	5	3	15
AC	15	8	11	4	36	27	26	24	36	35	36	113
AD	0	0	0	0	6	1	0	1	0	0	6	2
BB	32	30	36	12	14	0	0	0	1	0	0	0
BC	14	6	8	6	6	2	3	1	2	1	1	8
BD	0	1	0	0	0	1	0	0	1	0	0	2
BE	1	0	0	0	1	1	0	0	0	0	1	1
CB	0	1	0	0	0	0	0	0	0	0	0	0
CC	5	2	0	2	12	6	8	8	8	9	12	30
CD	0	0	0	0	1	0	0	0	0	0	1	0
DB	0	0	0	0	0	0	0	0	1	0	0	1
DC	1	1	0	1	2	1	1	0	0	3	2	2
EB	0	0	0	1	0	0	0	0	0	0	0	0
EC	0	0	1	0	1	2	0	1	1	1	1	4
H	0.716	0.623	0.553	0.749	0.753	0.640	0.601	0.659	0.568	0.566	0.634	0.604
n	71	51	57	27	82	46	43	44	56	55	63	188

fell into two highly divergent clades (I and II) that were unequally distributed between the Atlantic and Indo-Pacific. Their clade II genotype occurred at a much higher frequency in the Atlantic (65.2–74.4%) than in the Indo-Pacific (4.3–9.1%). These results are similar to those of the present study using samples of larger size and from a wider geographical area. However, the genetic marker in the *ATCO* segment in the present study appears to be more powerful for discriminating Atlantic and Indo-Pacific fishes.

Although Kume *et al*. (1971) assumed very little relationship between the Indian and Pacific bigeye tunas, genetic analyses in the present study and those of Alvarado-Bremer *et al*. (1998) and Suzuki (1962) all detected no differences between samples from the Indian and Pacific Oceans. Recent investigations on the physical structure of the Pacific and Indian Oceans have revealed a water pathway between the Pacific and Indian Oceans (Cresswell *et al*. 1993; Fieux *et al*. 1994; Meyers *et al*. 1995; Gordon & Fine 1996). The Indonesian throughflow along this pathway transports the north and south Pacific waters to the Indian Ocean through the complex system of the Australasian Mediterranean Seas, by which the bigeye larvae and young juveniles of the western tropical Pacific, a main spawning ground of bigeye tuna (Nishikawa *et al*. 1985), may be transported to the Indian Ocean. Furthermore, bigeye catch records by the Japanese longline fishery (Anonymous 1980) indicate a continuous distribution of adult and subadult bigeyes through the Halmahera, Seram, Banda and Timor Seas, suggesting an interchange of fish between the Pacific and Indian Oceans.

In contrast, the differences in the genotype distributions between Atlantic and Indo-Pacific samples are striking. Alvarado-Bremer *et al*. (1998) suggests some gene flow from the Atlantic to the Indo-Pacific because of the presence of the Atlantic genotypes in the Pacific. But the results found for the *ATCO* segment in the present study are definitive, and we can conclude that there is neither gene flow nor fish migration from the Atlantic to the Indian Ocean. However, fish migration and gene flow in the opposite direction may be possible. No heterogeneity of genotype distribution in the D-loop segment of β type individuals was observed among the Atlantic, Cape and Indo-Pacific samples, suggesting that migration or immigration by the Indo-Pacific bigeyes into the Atlantic is ongoing or has occurred recently. However, genotype frequencies both in the *ATCO* and D-loop segments are homogeneous in all samples collected throughout the Atlantic, strongly suggesting that the immigrants, if any, have not changed the mtDNA genotype frequencies in the Atlantic bigeye stock. This indicates that the frequency and number of immigrants from the Indian Ocean at the present time is small. Thus, a strong genetic break between the Atlantic and Indian stocks of bigeye tuna may exist around South Africa, where fishes from the two distinct stocks coexist.

The water transport between the Indian and Atlantic Oceans is summarized in Tomczak & Godfrey (1994). In the Indian Ocean, the south equatorial current bifurcates into the Mozambique and east Madagascar currents east of Madagascar. These currents merge south of Madagascar and feed the westward flowing Agulhas current. A large part of this current joins the south Indian Ocean current, by which adult and subadult bigeyes may return to the tropical and subtropical Indian Ocean. A part of the warm Agulhas current rounds the Cape of Good Hope, and is incorporated into the cool Benguela current as rings and eddies. Thus, the Agulhas current, one of the strongest currents in the world, may transport adult and subadult bigeyes of the Indian Ocean to the South Atlantic and may also prevent Atlantic bigeyes from penetrating into the Indian Ocean. However, ocean currents may not be the primary factor separating bigeye tuna stocks of the Atlantic and Indian Oceans, as catch records (data not shown) of the Cape samples indicate that both α and β type fishes have been caught on the same day and at the same location. Furthermore, the Cape-6 sample was from 15° east, well inside the South Atlantic, but the genotype distributions in the *ATCO* segment indicate this sample had greater affinity to the Indo-Pacific samples. This might be explained by the fact that the Cape-6 sample was caught in the retroflection region of the Agulhas current (see Tomczak & Godfrey (1994)). The distribution and mixture of fishes from each stock must be affected by the dynamics of the currents around South Africa. Thus, there appears to be no physical barrier preventing bigeye tunas from mutual penetration. Hence, it is likely that bigeye tuna return to their oceans of origin, just like salmon, for reproduction.

The accurate estimation of a population mixture is a primary component of fishery management. The apparent genetic break between Atlantic and Pacific stocks of bigeye tuna and the very simple but diagnostic marker found in this study may simplify the estimation of the mixing ratio in the catch. Given that genetic isolation between the Atlantic and Indo-Pacific stocks is complete and there is no genetic structuring within each ocean basin, frequencies of the α type individual in the standard populations of Atlantic and Indo-Pacific were simply calculated to be 0.730 (A) and 0.005 (P), respectively. The mixing ratio (θ) of Atlantic bigeye tuna in a sample can be calculated by the formula; $n/N = \theta A + (1 - \theta)P$, where N is the sample size and n is the number of α type individuals in a sample. The mixing ratio of Atlantic bigeye tuna in the pooled Cape sample was then estimated to be 0.29, and that in each Cape sample ranged from 0.04 in Cape-7 to 1 in Cape-3.

Large marine pelagic fishes such as tuna and billfishes

may have a homogeneous genetic population structure on a large geographical scale, as they are highly migratory with a very wide reproductive area and have extensive egg and larval dispersal in ocean currents. Nevertheless, recent advances in molecular genetic methods have allowed us to analyse large sample sets with higher sensitivity, consequently detecting small to large signals of population subdivision in several species. Chow *et al*. (1997) performed RFLP analysis on the mtDNA D-loop region of the swordfish (*Xiphias gladius*) and revealed genetic distinctness of the Mediterranean stock. High levels of polymorphism by *Rsa*I digestion were observed in 12 samples collected world-wide, but the Mediterranean sample was completely monomorphic, indicating that exogenous swordfish rarely enter the Sea. The relationships between the Mediterranean stock and colleagues may be similar to the case in the bigeye tuna. However, high levels of variation in the other swordfish samples may make the estimation of stock mixing much less accurate than in bigeye tuna. Nucleotide sequence analysis of the D-loop segment also indicated the genetic uniqueness of the Mediterranean swordfish stock (Rosel & Block 1995), but many rare genotypes produced by sequence analysis did not increase the resolving power or may even have obscured existing differences between stocks. RFLP analysis on the *ATCO* segment of albacore (*Thunnus alalunga*) yielded seven composite genotypes among 620 individuals examined (Chow & Ushiama 1995), and the heterogeneous distribution of the genotypes indicated population subdivision between the Atlantic and Pacific Oceans. They also suggested the mixture of fishes from different stocks around South Africa. But the genetic marker may not be powerful enough to estimate accurately the mixing ratio, as all samples shared common genotypes.

Thus, highly polymorphic genetic markers are potentially capable of detecting subtle signals of population subdivision on small spatial scales and over short periods of time, but may not be practical for accurately estimating the stock mixing ratio because of the inherent large variance. In contrast, the simple genetic marker found in the present study is nearly ideal. Spatio-temporal sampling of bigeye tuna around South Africa followed by genetic analysis using this marker will reveal more about the extent and dynamics of population mixing around South Africa, and this information may be critical for a proper and practical assessment of mixing in the catch.

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This paper is part of our research in stock assessment and management in highly migratory tuna and billfish species. S. Chow is a senior researcher working mainly on molecular genetics. H. Okamoto and N. Miyabe are also senior researchers dealing with tuna stock assessment and statistics, as well as biological investigation. K. Hiramatsu is a mathematician, estimating stock mixing ratios. N. Barut is from the Bureau of Fisheries and Aquatic Resources and has managed fish sample collections in Philippine waters.
