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RESEARCH ARTICLE

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Nuclear and mitochondrial DNA markers indicate unidirectional gene flow of Indo-Pacific to Atlantic bigeye tuna (*Thunnus obesus*) populations, and their admixture off southern Africa

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Abstract A sharp genetic break separates Atlantic from Indo-Pacific bigeye tuna (*Thunnus obesus*) populations, as the frequencies of two major mitochondrial (mt) DNA types (α and β) found in this species are different across the tip of southern Africa. The level of nucleotide divergence between mtDNA types α and β is of the same order as that between reproductively isolated taxa. To further investigate the genetic structure of bigeye tuna over its distribution range and in the contact zone off southern Africa, bigeye tuna samples collected between 1992 and 2001 (including samples from a previous mtDNA survey) were characterized for four nuclear DNA loci and for mtDNA. Nuclear markers did not support the hypothesis that α and β mitochondria characterize sibling species. Significant allele-frequency differences at one intronic locus (*GH2*) and one microsatellite locus (μ208) were found between Atlantic and Indo-Pacific samples, although the level of nuclear genetic differentiation (Weir and Cockerham's $\hat{\theta}=0.025$ to 0.042) was much lower than in mtDNA ($\hat{\theta}=0.664$ to 0.807). Probabilistic Bayesian assignment of individuals to a population confirmed that southern African bigeye tuna samples represent a simple mixture of individuals from Atlantic and Indian stocks that do not interbreed, with a higher contribution from Indian Ocean individuals (about 2/3 vs. 1/3).

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Introduction

Defining population genetic structure in species under intense fishing pressure is desirable for both management and conservation. As different genetic markers may show different responses to gene flow, selection and genetic drift, assessing population structure may require complementary sets of markers, such as mitochondrial (mt) and non-coding nuclear (n) DNA (Ward 2000). Among marine species undergoing large harvests worldwide, abundant population genetic data are already available for tunas (Chow and Ushiama 1995; Alvarado Bremer et al. 1998; Chow et al. 2000; Appleyard et al. 2002a; Ely et al. 2002; Viñas et al. 2004, and references therein). Genetic differentiation has generally been supposed to be low among tuna populations within and between oceans. This is presumably related to the biological characteristics of tuna species, such as reproduction in the open ocean, large effective population sizes, and high adult vagility.

Bigeye tuna (*Thunnus obesus*), a commercially highly prized species, shows significant differences in mtDNA-haplotype frequencies between populations of the Atlantic and the Indo-Pacific Oceans, while no genetic structure is evident within an ocean, demonstrating extensive gene flow at intra-oceanic scales (Alvarado Bremer et al. 1998; Grewe and Hampton 1998; Chow et al. 2000; Appleyard et al. 2002a). Analysis of the mtDNA control region revealed two major, highly divergent clades in bigeye tuna: clade I is nearly fixed in the Indo-Pacific, whereas clade II is dominant in the Atlantic, where it coexists with clade I (Alvarado Bre-

mer et al. 1998). Chow et al. (2000) used a fragment of the flanking region between the ATPase-6 and cytochrome oxidase subunit III genes [the *ATCO* fragment of Chow and Inoue (1993)] as genetic marker, and found that the Indo-Pacific population of *T. obesus* was nearly fixed for haplotype "β", whereas the Atlantic population was dominated by haplotype "α". MtDNA types α and β correspond to clades II and I of Alvarado Bremer et al. (1998), respectively, and mtDNA types BET2 and BET1 of Appleyard et al. (2002a), respectively. Nucleotide sequence divergence (estimated from Tamura–Nei's distances) for the control region between clades I and II was $4.9 \pm 0.1\%$ (Alvarado Bremer et al. 1998), and it was 1.6–2.1% for the *ATCO* fragment (Takeyama et al. 2001). Similar patterns and levels of mtDNA divergence have been reported in blue marlin (*Makaira nigricans*, Buonaccorsi et al. 2001), and sailfish (*Istiophorus platypterus*, Graves and McDowell 2003).

It has been proposed that the sharp differentiation between the Atlantic and Indo-Pacific mitochondrial lineages of bigeye tuna, blue marlin, and sailfish resulted from Pleistocene vicariance (Alvarado Bremer et al. 1998; Buonaccorsi et al. 2001; Graves and McDowell 2003). During colder periods, water temperature was presumably too low for migration of tropical fish around the tip of southern Africa, whereas the Indian and Pacific Oceans remained effectively connected. With subsequent warming, the distribution area of bigeye tuna expanded southward to off southern Africa, where contact was re-established between the two populations. There is no indication that the waters around southern Africa are breeding areas for bigeye tuna, since no larvae or female fish with mature ovaries have ever been collected in this region (Nishikawa et al. 1985). A major oceanic front is present off southern Africa, which is transiently used as a feeding ground by bigeye tuna of both oceans (Kirby et al. 2000). Patterns of both historical and present-day migration of individuals from one ocean to the other are still poorly known. For instance, Chow et al. (2000) reported that the Atlantic α (clade II/BET2) mtDNA type was absent from the Indian Ocean, and suggested the absence of migrants from the Atlantic to the Indo-Pacific (but see Alvaredo-Bremer et al. 1998). In a more recent survey, Appleyard et al. (2002a) reported that BET2 haplotypes were present at low frequency in the Indo-Pacific [4/276 (1.45%) individuals analyzed; all from western Indian collections]. This suggested possible migration of individuals from the Atlantic into the Indo-Pacific. Conversely, the occurrence at high frequency, in Atlantic populations, of the Indo-Pacific β (clade I/BET1) mtDNA type may indicate strong historical and/or present-day gene flow from the Indo-Pacific into the Atlantic.

Since mtDNA is uniparentally inherited, its effective population size is smaller than that of nuclear genes (Palumbi and Baker 1994; FitzSimmons et al. 1997). MtDNA is therefore more sensitive to demographic events that may have affected genetic variation, such as reduction in population size and geographic isolation. As mtDNAs do not recombine, a mixture of haplotypes at a location can reflect either simple temporary admixture of individuals from the two populations (the hypothesis favored by Chow et al. 2000), interbreeding, or the presence of another independent population. It is necessary, therefore, to further investigate the population genetics of bigeye tuna using nuclear gene markers. Alvarado Bremer et al. (1998) and Takeyama et al. (2001) pointed out that nucleotide divergence between clades I and II [or β and α mtDNA types of Chow et al. (2000), respectively] was of the same order as the divergence between yellowfin (*Thunnus albacares*), blackfin (*T. atlanticus*), and longtail (*T. tonggol*) tunas, suggesting that the two clades might represent two subspecies or even two species. If the assumption of two species is true (i.e. a particular mitochondrial clade exclusively associated to a species), the nuclear backgrounds of the α/clade II and β/clade I types within the Atlantic Ocean, where they coexist, should also be differentiated. Secondly, if the patterns of genetic differentiation prove dissimilar between mitochondrial and nuclear genes, it would be important to consider more thoroughly how nuclear genetic variation is structured across and within oceans, and how populations mix in the presumed contact zone off southern Africa. Simple admixture [referred to as "mechanical mixing" by Nielsen et al. (2003)] should leave its signature in the form of heterozygote deficiencies and genotypic disequilibria across loci that differ in allele frequency between the Indo-Pacific and Atlantic populations (Wahlund effect). If the two populations interbreed, then no heterozygote deficiency should be present, even though one may observe genotypic disequilibria across loci when contact between populations is sufficiently recent.

In the present study we used nuclear and mitochondrial DNA markers: (1) to test the hypothesis of two bigeye tuna species corresponding to mitochondrial types α/clade II and β/clade I, (2) to assess the level of inter-oceanic divergence, and (3) to test the admixture hypothesis in the presumed contact zone off southern Africa.

Materials and methods

Sampling

A total of 15 bigeye tuna (*Thunnus obesus*) samples of 5 to 51 individuals (total $N = 339$) were collected between 1992 and 2001 in the Atlantic, Indian, and Pacific Oceans (Table 1). These samples were obtained from scientific cruises and commercial fishing operations, and consisted of small pieces of muscle preserved either dried, frozen, or in ethanol. Samples from the Atlantic (CNA and NWA), southern Africa (Cape-1 to Cape-7), Indian Ocean (IBE), and eastern Pacific (Peru) were used previously (Chow et al. 2000). A central Atlantic sample

Table 1 *Thunnus obesus*. Details on sampling locations and dates. Samples CNA, NWA, Cape-1 to Cape-7, and Peru were from the collections of the National Research Institute of Far Sea Fisheries (Shimizu, Japan); sample Tob was collected by long line during cruise SY0002 of R.V. "Shoyo Maru"; sample IBE was purchased from a fish landing sites at Makurazaki Fish Port in Japan, but was caught by purse seine south of Sri Lanka; samples S, Mad, and S2 were collected by CSIRO and IRD observers on board commercial vessels (*na* not available; *N* sample size)

Oceanic region, area	(*N*)	Code	Latitude	Longitude	Date
Atlantic Ocean					
Central North	(50)	CNA	07–09°N	27–28°W	Jan–Feb 1997
Central North	(7)	Tob	10–21°N	27–36°W	Oct 2000
North West	(50)	NWA	37–41°N	48–67°W	Apr–May 1997
Southern Africa					
Off Cape of Good Hope	(12)	Cape-1	38–41°S	24–25°E	May–Jun 1992
Off Cape of Good Hope	(5)	Cape-2	na	na	Nov 1997
Off Cape of Good Hope	(10)	Cape-3	na	na	Dec 1997
Off Cape of Good Hope	(10)	Cape-4	na	na	Sep1998
Off Cape of Good Hope	(7)	Cape-5	na	na	Sep 1998
Off Cape of Good Hope	(21)	Cape-6	40°S	15°E	Aug 1998
Off Cape of Good Hope	(33)	Cape-7	40°S	25°E	Aug 1998
Indian Ocean					
Sri Lanka	(50)	IBE	na	na	1996
Seychelles	(16)	S	00–11°S	50–88°E	Dec 1998–Jan 1999
Seychelles	(12)	S2	02–04°S	46–57°E	Oct 2001
Madagascar	(5)	Mad	na	na	Feb 1999
Eastern Pacific	(51)	Peru	08–17°S	84–124°W	Jun 1994

(Tob) was collected during research cruise SY002 of R.V. "Shoyo Maru" conducted by the Fisheries Agency of Japan. Samples from the Seychelles (S and S2) and Madagascar (Mad) were kindly provided by Dr R.D. Ward (CSIRO, Hobart).

Genetic analyses

Most samples were analyzed for genetic variation at four nDNA loci, including a microsatellite locus (*cmrTa-208*, subsequently referred to as µ208), the first intron of the alpha-amylase gene locus (*Amyl*), the second intron of the growth hormone locus (*GH2*), and one anonymous locus (*Anom231*). Protocols and data for the mtDNA *ATCO* segment were from Chow et al. (2000), and the same protocols were applied to the other samples used in the present survey. The analysis of allele-size variation at locus µ208 followed those of Grewe et al. (2000). *Amyl* was PCR-amplified using primers *Am2b1F* and *Am2b2R* of Hassan et al. (2002). Locus *Anom231* was selected on the basis of apparent Mendelian inheritance on polyacrylamide gels using non-specific PCR products from direct amplification of length polymorphisms (DALP-PCR: Desmarais et al. 1998). Specific primers *Anom231F* (5′-TGTAAGGTTCTTTAGTTCCAG-3′) and *Anom231R* (5′-TCAAGGTGCAACTAATTCAC-3′) were then designed from the alignment of sequences of allelic PCR products excised from the gels and separately re-amplified. Primer pairs used for the amplification of alleles at locus *GH2* [respectively, *GH2F* (5′-CAACACCTCCACCTGCTCGC-3′)/*GH2R* (5′-CCTG CAGGAAGATTTTGTTG-3′)] were designed from the alignment of the complete sequence of the growth hormone gene of *Lates calcarifer* (GenBank U16816), with the homologous cDNA sequences of *L. calcarifer* (GenBank X59378), *Thunnus* sp. (GenBank X06735), *Sciaenops ocellatus* (GenBank AF065165), and *Lateolabrax japonicus* (GenBank L43629). Individual PCR amplifications at loci *GH2*, *Amyl*, and *Anom231* were carried out in 10 µl reaction mixture containing 1 µl DNA template, 0.4 µM of each primer, with the 5′-end of one primer per set labeled with either fluorochrome CY5, TAMRA, or 6-FAM (Eurogentec, Strasbourg, France), 1.5 mM $MgCl_2$ (Promega, Madison, Wis., USA), 74 µM of each dNTP, and 0.25 U *Taq* polymerase (Promega, Madison, Wis., USA). Amplification conditions were 35 cycles of denaturation at 94°C for 30 s, annealing at 48°C (*Amyl*), 50°C (*Anom231*), or 52°C (*GH2*) for 30 s, and extension at 72°C for 30 s. Amplification products were used undiluted and mixed with an equal volume of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% xylene cyanol, and 0.05% bromophenol blue), denatured at 94°C for 5 min. Then, 3 µl of this mixture was loaded into 6% denaturing polyacrylamide gel and run using 1× TBE buffer at 50 W for 45 min (µ208), 2 h (*GH2*), 2.5 h (*Anom231*), or 4 h (*Amyl*). The gels were then laser-scanned at 505 nm (6-FAM), 585 nm (TAMRA), and 675 nm (CY5), and the respective 6-FAM, TAMRA, and CY5 fluorescent bands were visualized in an FMBio II fluorescence imaging apparatus (Hitachi Instruments, San José, Calif., USA). Several individuals of known genotype were used as additional allele-size standards on each gel.

Statistical analysis

Gene diversity at a locus (*h*) was estimated from genotypic frequencies using the Genetix v.4.0 software (Belkhir et al. 2000). Deviations from Hardy–Weinberg expectations within samples were investigated using Weir and Cockerham's (1984) $\hat{f}$. The null hypothesis of no significant departure from Hardy–Weinberg expectations ($f=0$) was tested by randomly permutating alleles from the original matrix of genotypes with Genetix v.4.0. Levels of population differentiation were investigated using Weir and Cockerham's (1984) $\hat{\theta}$, an estimator of Wright's (1951) F_{st}, also using Genetix

v.4.0. Linkage disequilibrium between pairs of nuclear loci was tested with the software Genepop (Raymond and Rousset 1995).

Population assignment

We used the probabilistic Bayesian assignment test implemented in the Structure v.2.0 program of Pritchard et al. (2000) to assign bigeye tuna taken off southern Africa (total sample size, $N = 84$) to their population of most likely origin. Once their genetic characteristics were assessed (no departure from Hardy–Weinberg equilibrium; no departure from linkage equilibrium), Atlantic (CNA) and Indian (IBE) samples were considered as representing ''baseline'' or reference populations used to assign ''unknowns'' (the seven Cape samples collected off southern Africa; Table 1). We first performed assignment on the whole data set of bigeye tuna from southern Africa: (1) considering all loci and (2) considering the mtDNA and only those nuclear loci with significant allele-frequency differences between baseline populations. Subsequently, we performed assignments using all loci for each of the southern African subsamples (Cape-1 to Cape-7). The latter analyses were done on those individuals genotyped for at least two nuclear loci. The probability of assignment of each individual was computed as the posterior probability to belong to the Indian population. Arbitrarily, we considered an individual as originating from the Indian population if its posterior probability was >0.95. Alternatively, an individual with a posterior probability P[Indian origin]<0.05 was considered as being of Atlantic origin ($1-P$[Indian origin]$<0.05=$ P[Atlantic origin]>0.95). Distributions of posterior probabilities may indicate whether southern African samples were mainly of Indian ($P>0.5$) or Atlantic ($P<0.5$) origin, thus providing a rough estimate of admixture proportions in samples.

The hypothesis of simple admixture versus interbreeding in the populations sampled off southern Africa was tested using a method developed by Nielsen et al. (2003), slightly modified to accommodate mtDNA data. We used the allelic frequency profiles of Indian (IBE) and Atlantic (CNA) baseline populations to build a hybrid pseudo-sample of the same size as the Cape sample. This procedure simulates interbreeding between Atlantic and Indian tunas, with respect to the admixture proportions that were independently estimated (1/3 Atlantic, 2/3 Indian). For nuclear loci, we created simulated hybrid genotypes by drawing alleles randomly from the observed allele frequency distribution in each baseline population. For the mtDNA locus, we assigned at random one haplotype or the other (α or β) to those simulated individuals, according to haplotype frequencies in the baseline populations. We used the Kolmogorov–Smirnov two-sample test (Sokal and Rohlf 1995) to evaluate differences between the observed distribution of individual assignments for the Cape sample and the distribution in the simulated Cape sample.

Results

Not all samples were screened at nuclear loci due to unsuccessful PCR amplification or non-repeatable band patterns in some samples (NWA, Tob, S, S2, and Mad; Table 2). However, genetic data were completed at all loci for Atlantic (CNA), Indo-Pacific (IBE and Peru), and southern African (Cape-1 to Cape-7) samples (Table 2).

Gene diversity, Hardy–Weinberg, and linkage equilibria

At locus µ208 20 alleles were scored ($h=0.64$–0.93), at *GH2* 15 ($h=0.50$–0.78), at *Amyl* 4 ($h=0.32$–0.65), and at *Anom231* 4 ($h=0.00$–0.33). Allele frequencies at these nuclear loci and haplotype frequencies at the *ATCO* locus are shown in Table 2. No significant deviation from Hardy–Weinberg proportions was observed at any nuclear locus in individual samples after Bonferroni correction (Table 2). When all Cape samples were pooled, significant heterozygote deficiencies were observed at loci *GH2* and µ208 (Table 2), indicating a Wahlund effect.

No linkage disequilibrium was detected within populations from the Atlantic, Indian, and Pacific Oceans. When all samples taken off southern Africa were pooled, linkage disequilibrium was apparent for 2 of 15 pairwise comparisons (*ATCO*–*GH2*: $P=0.028$; *ATCO*–µ208: $P=0.021$). These were for pairs of loci that both exhibited allele-frequency differences between Indian and Atlantic populations (see below; Table 3). Disequilibria between the same pairs of loci were also observed within Cape-1 to Cape-7 samples, but were not statistically significant, perhaps because of low sample sizes (results not shown).

Population structure

Since no significant allele-frequency differences were found between samples from the same area (CNA/Tob and S/S2/Mad), these were pooled for further analysis, as were Cape-1 to Cape-7 samples. Highly significant mtDNA genetic differences were observed between Atlantic and Indian or Pacific Ocean samples ($\hat{\theta}=0.710$ to 0.807; Table 3), but no significant differences in haplotype frequencies were observed between samples within an ocean, nor between the Indian and Pacific samples. Significant genetic differences were found at nuclear locus *GH2* between the Atlantic population and Indo-Pacific populations IBE and Peru ($\hat{\theta}=0.062$ to 0.093), although not with another group of samples from the Indian Ocean (S/S2/Mad) ($\hat{\theta}=0.018$ to 0.025). No significant heterogeneity was found within the

Table 2 *Thunnus obesus*. Allele frequencies and estimates of population genetic parameters at one mitochondrial and four nuclear DNA loci, in 15 samples from the Atlantic and the Indo-Pacific. Multiple-locus parameters were estimated as averages across loci. Sample codes as in Table 1 [*N* sample size; *n* number of alleles at a locus; *h* gene diversity at a locus; *H* average of *h* across loci; $\hat{f}$ estimate of Weir and Cockerham's (1984) equivalent of Wright's fixation index; *nuclear* average values across all four nuclear loci (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)]. *Bold* values significant after Bonferroni correction (Sokal and Rohlf 1995)

Locus, allele	NWA	Tob	CNA	Cape-1	Cape-2	Cape-3	Cape-4	Cape-5	Cape-6	Cape-7	S	S2	Mad	IBE	Peru	Cape 1–7
ATCO																
α	0.72	0.80	0.80	0.17	0.60	0.80	0.10	0.29	0.16	0.04	0	0	0	0	0.02	0.22
β	0.28	0.20	0.20	0.83	0.40	0.20	0.90	0.71	0.84	0.96	1	1	1	1	0.98	0.78
(*N*)	(40)	(5)	(46)	(12)	(5)	(10)	(10)	(7)	(19)	(28)	(16)	(3)	(4)	(50)	(57)	(91)
n	2	2	2	2	2	2	2	2	2	2	1	1	1	1	2	2
h	0.409	0.400	0.322	0.304	0.600	0.356	0.200	0.476	0.281	0.072	0	0	0	0	0.036	0.344
GH2																
098	0.33	0.38	0.31	0.14	0.60	0.14	0.05	0.36	0.11	0.13	0.17	0.07	0	0.06	0.03	0.16
100	0.11	0.12	0.11	0.04	0.10	0.07	0.10	0	0.08	0.06	0.17	0.21	0	0.08	0.03	0.07
101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0
102	0.35	0.38	0.34	0.46	0.10	0.57	0.55	0.14	0.50	0.56	0.46	0.50	0.25	0.52	0.48	0.46
103	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0	0
104	0.18	0.12	0.23	0.36	0.20	0.22	0.30	0.36	0.28	0.19	0.20	0.22	0.75	0.32	0.31	0.26
105	0	0	0	0	0	0	0	0	0	0.02	0	0	0	0.01	0	0.01
106	0.03	0	0	0	0	0	0	0.07	0	0	0	0	0	0	0.04	0.01
107	0	0	0.01	0	0	0	0	0	0.03	0	0	0	0	0	0	0.01
108	0	0	0	0	0	0	0	0.07	0	0	0	0	0	0	0.01	0.01
109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0
110	0	0	0	0	0	0	0	0	0	0.04	0	0	0	0	0.05	0.01
114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0
116	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0
120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0
(*N*)	(36)	(4)	(50)	(11)	(5)	(7)	(10)	(7)	(18)	(24)	(12)	(7)	(2)	(50)	(60)	(82)
n	5	4	5	4	4	4	4	5	5	6	4	4	2	6	12	9
h	0.733	0.786	0.731	0.671	0.644	0.648	0.626	0.769	0.671	0.640	0.721	0.703	0.500	0.623	0.673	0.683
$\hat{f}$	0.091	0.400	0.097	−0.088	0.407*	−0.111	−0.297	0.077	0.177	0.354**	0.433*	−0.241	0.000	0.135	0.034	0.144*
μ208																
138	–	–	0.02	0	0	0	0.06	0	0	0	–	–	–	0	0.03	0.01
140	–	–	0.00	0	0	0	0	0	0	0	–	–	–	0	0.07	0
142	–	–	0.04	0	0	0	0	0.07	0	0.04	–	–	–	0.01	0.05	0.02
144	–	–	0.01	0	0	0	0	0	0	0	–	–	–	0.02	0.01	0
146	–	–	0.03	0	0	0	0	0	0	0	–	–	–	0.01	0.03	0
148	–	–	0.32	0.20	0.63	0.13	0.16	0.14	0.22	0.10	–	–	–	0.29	0.15	0.18
150	–	–	0.16	0.50	0.13	0.13	0.50	0.29	0	0.39	–	–	–	0.32	0.40	0.30
152	–	–	0.09	0.05	0	0	0.16	0.21	0.46	0.25	–	–	–	0.16	0.16	0.21
154	–	–	0.10	0.05	0.12	0.25	0.06	0.29	0.04	0.10	–	–	–	0.06	0.03	0.10
156	–	–	0.09	0.10	0.12	0.25	0	0	0.08	0.04	–	–	–	0.04	0.03	0.07
158	–	–	0.09	0.05	0	0.12	0.06	0	0.08	0	–	–	–	0.02	0.01	0.04
160	–	–	0.01	0	0	0.12	0	0	0	0	–	–	–	0	0.01	0.01
162	–	–	0.01	0	0	0	0	0	0	0.04	–	–	–	0.01	0	0.01
164	–	–	0.01	0	0	0	0	0	0	0	–	–	–	0.01	0.01	0
166	–	–	0.01	0	0	0	0	0	0.04	0.04	–	–	–	0.01	0	0.02
168	–	–	0	0.05	0	0	0	0	0.04	0	–	–	–	0.01	0.01	0.02
170	–	–	0	0	0	0	0	0	0	0	–	–	–	0.01	0	0
172	–	–	0	0	0	0	0	0	0.04	0	–	–	–	0.01	0	0.01
174	–	–	0	0	0	0	0	0	0	0	–	–	–	0.01	0	0
176	–	–	0.01	0	0	0	0	0	0	0	–	–	–	0	0	0
(*N*)	–	–	(45)	(10)	(4)	(4)	(9)	(7)	(12)	(14)	–	–	–	(43)	(38)	(60)
n	–	–	15	7	4	6	6	5	8	8	–	–	–	16	14	13
h	–	–	0.850	0.726	0.643	0.929	0.726	0.824	0.757	0.783	–	–	–	0.793	0.794	0.818
$\hat{f}$	–	–	0.086	0.182	0.250	0.217	0.402	0.500*	−0.106	0.370**	–	–	–	–0.146	0.106	**0.249***
Amyl																
070	–	–	0	0	0	0	0	0	0	0.04	–	–	–	0.02	0	0.01
090	–	–	0	0	0	0	0	0.14	0	0	–	–	–	0.01	0.01	0.02
100	–	–	0.67	0.75	0.50	0.50	0.67	0.50	0.81	0.75	–	–	–	0.66	0.69	0.69
110	–	–	0.33	0.25	0.50	0.50	0.33	0.36	0.19	0.21	–	–	–	0.31	0.30	0.28
(*N*)	–	–	(48)	(6)	(4)	(5)	(9)	(7)	(16)	(12)	–	–	–	(50)	(39)	(59)
n	–	–	2	2	2	2	2	3	2	3	–	–	–	4	3	4
h	–	–	0.449	0.409	0.571	0.556	0.471	0.648	0.315	0.400	–	–	–	0.473	0.446	0.449
$\hat{f}$	–	–	−0.021	0.615	0.143	−0.091	−0.455	0.357	−0.200	−0.234	–	–	–	−0.016	0.016	−0.019
Anom231																
080	-	-	0.01	0	0	0	0	0	0	0	-	-	-	0	0.02	0

Table 2 (Contd.)

Locus, allele	NWA	Tob	CNA	Cape-1	Cape-2	Cape-3	Cape-4	Cape-5	Cape-6	Cape-7	S	S2	Mad	IBE	Peru	Cape 1–7
090	-	-	0.06	0	0.17	0	0.11	0.07	0.11	0	-	-	-	0.04	0.01	0.06
095	-	-	0	0	0	0	0.06	0	0.04	0	-	-	-	0	0	0.02
100	-	-	0.93	1	0.83	1	0.83	0.93	0.85	1	-	-	-	0.96	0.97	0.92
(*N*)	-	-	(49)	(10)	(3)	(4)	(9)	(7)	(13)	(18)	-	-	-	(50)	(49)	(63)
n	-	-	3	1	2	1	3	2	3	1	-	-	-	2	3	3
h	-	-	0.135	0.000	0.333	0.000	0.307	0.143	0.280	0.000	-	-	-	0.078	0.058	0.136
$\hat{f}$	-	-	−0.057	-	0.000	-	−0.091	0.000	0.182	-	-	-	-	−0.032	−0.014	0.063
Nuclear																
n	–	–	6.25	3.5	3	3.25	3.75	3.75	4.5	4.5	–	–	–	7	8	7
H	–	–	0.541	0.452	0.548	0.533	0.532	0.596	0.506	0.458	–	–	–	0.492	0.493	0.52
$\hat{f}$	–	–	0.059	0.186	0.232	0.040	-0.057	0.297*	0.014	0.232**	–	–	–	−0.021	0.058	0.130**

Indian Ocean ($\hat{\theta}=-0.008$ to 0.004) or within the Atlantic Ocean ($\hat{\theta}=-0.011$). Significant genetic differentiation was also demonstrated at locus $\mu 208$ between Atlantic and Indo-Pacific samples ($\hat{\theta}=0.016$ to 0.056; Table 3). No significant differences were apparent between oceans for *Anom231* or *Amyl*. Overall, nuclear loci exhibited substantially lower levels of inter-oceanic genetic differentiation than mtDNA.

Table 3 *Thunnus obesus*. Pairwise $\hat{\theta}$-values at the *ATCO* locus and at four nuclear DNA loci [*nuclear* multiple-locus $\hat{\theta}$-estimates across all four nuclear loci; *overall* multiple-locus $\hat{\theta}$-estimates across all five loci (*$P<0.05$, **$P<0.01$, ***$P<0.001$)]. *Bold* values show significance after Bonferroni correction (Sokal and Rohlf 1995)

Locus, sample	CNA/Tob	Cape 1–7	S/S2/Mad	IBE	Peru
ATCO					
NWA	−0.006	**0.405*****	**0.664*****	**0.742*****	**0.710*****
CNA/Tob		**0.498*****	**0.748*****	**0.807*****	**0.780*****
Cape 1–7			0.127**	**0.166****	**0.133*****
S/S2/Mad				0.000	−0.018
IBE					−0.000
GH2					
NWA	−0.011	0.027*	0.025	**0.075*****	**0.093*****
CNA/Tob		0.021*	0.018	**0.062****	**0.079*****
Cape 1–7			−0.008	0.004	0.013
S/S2/Mad				−0.005	0.013
IBE					−0.005
µ208					
NWA	–	–	–	–	–
CNA/Tob		0.021*	–	0.016*	**0.056****
Cape 1–7			–	−0.000	0.005
S/S2/Mad				–	–
IBE					0.011
Anom231					
NWA	–	–	–	–	–
CNA/Tob		−0.008	–	−0.002	0.012
Cape 1–7			–	−0.003	0.012
S/S2/Mad				–	–
IBE					0.000
Amyl					
NWA	–	–	–	–	–
CNA/Tob		−0.006	–	−0.009	−0.010
Cape 1–7			–	−0.008	−0.010
S/S2/Mad				–	–
IBE					−0.010
Nuclear					
NWA	–	–	–	–	–
CNA		0.013*	–	**0.025****	**0.042*****
Cape 1–7			–	−0.000	0.004
S/S2/Mad				–	–
IBE					0.001
Overall					
NWA	–	–	–	–	–
CNA		**0.130*****	–	**0.239*****	**0.239*****
Cape 1–7			–	**0.018****	**0.019****
S/S2/Mad				–	–
IBE					0.001

As two deeply divergent mtDNA clades have been observed in bigeye tuna (Alvarado Bremer et al. 1998; Chow et al. 2000), we further tested whether the nuclear background of individuals carrying α mitochondria was distinct from individuals carrying β mitochondria. This tests for the presence of two reproductively isolated forms, namely α and β. This analysis was carried out using the Atlantic samples (CNA, NWA and Tob), because here the two mitochondrial types were sympatric (Table 2). Atlantic bigeye tuna that carry α mitochondria were not significantly different from those carrying β mitochondria, either when using all nuclear loci (using sample CNA: $\hat{\theta}=0.007$; NS) or locus *GH2* alone (all Atlantic samples: $\hat{\theta}=-0.036$; NS). Thus, there was no correlation between mitochondrial haplotype and nuclear genomic background. This does not support the hypothesis of two reproductively isolated forms differing by their mitochondrial type. In contrast, significant differences were observed between Atlantic and Indo-Pacific individuals carrying β mitochondria (considering all nuclear loci: $\hat{\theta}=0.069$; $P<0.01$).

The pooled Cape sample was different from both the Atlantic and Indo-Pacific samples (Table 3). This, in addition to the significant heterozygote deficiencies observed in the pooled Cape sample (Table 2), underlines the heterogeneous composition of the populations sampled off southern Africa.

Assignment of southern African individuals

A larger proportion of southern African bigeye tuna was assigned to the Indo-Pacific than to the Atlantic population ($P>0.5$, $N=58$ vs. $P<0.5$, $N=28$ for the all-locus case; Fig. 1). Rough estimates of stock proportions into the Cape sample suggested a ratio of one-third of Atlantic-like bigeye tuna and two-thirds of Indian-like bigeye tuna (Fig. 1). Subsamples Cape-2 and Cape-3 were mainly composed of Atlantic-like bigeye tuna, whereas subsamples Cape-1, Cape-4, and Cape-7 were mainly composed of Indian-like bigeye tuna (Fig. 2). The other subsamples appeared to represent more balanced mixtures (Fig. 2). Individuals "with intermediate genotypes" (intermediate *P*-values) were uncommon (Figs. 1, 2), supporting the hypothesis of simple admixture of populations rather than the hypothesis of interbreeding. Only 22 out of 84 individuals had intermediate *P*-values ($0.10<P<0.90$; Fig. 1). To test the hypothesis of interbreeding more thoroughly, the

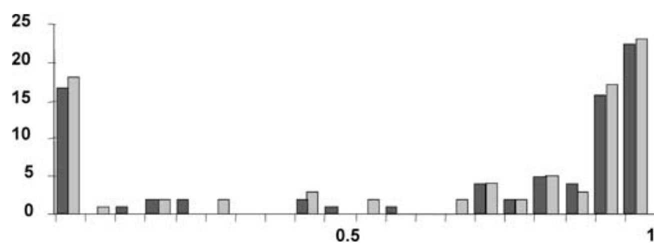

Fig. 1 *Thunnus obesus*. Posterior probability of assignment of Cape individuals to the Atlantic ($P<0.05$) or Indian ($P>0.95$) populations. Results are expressed as the number of individuals within fixed bins of the posterior probability [*dark bars* assignment based on all loci available (sample size, $N=84$); *light bars* assignment based on the three loci (μ208, *GH2*, *ATCO*) that showed significant allele frequency differences between Atlantic and Indian populations ($N=80$)]

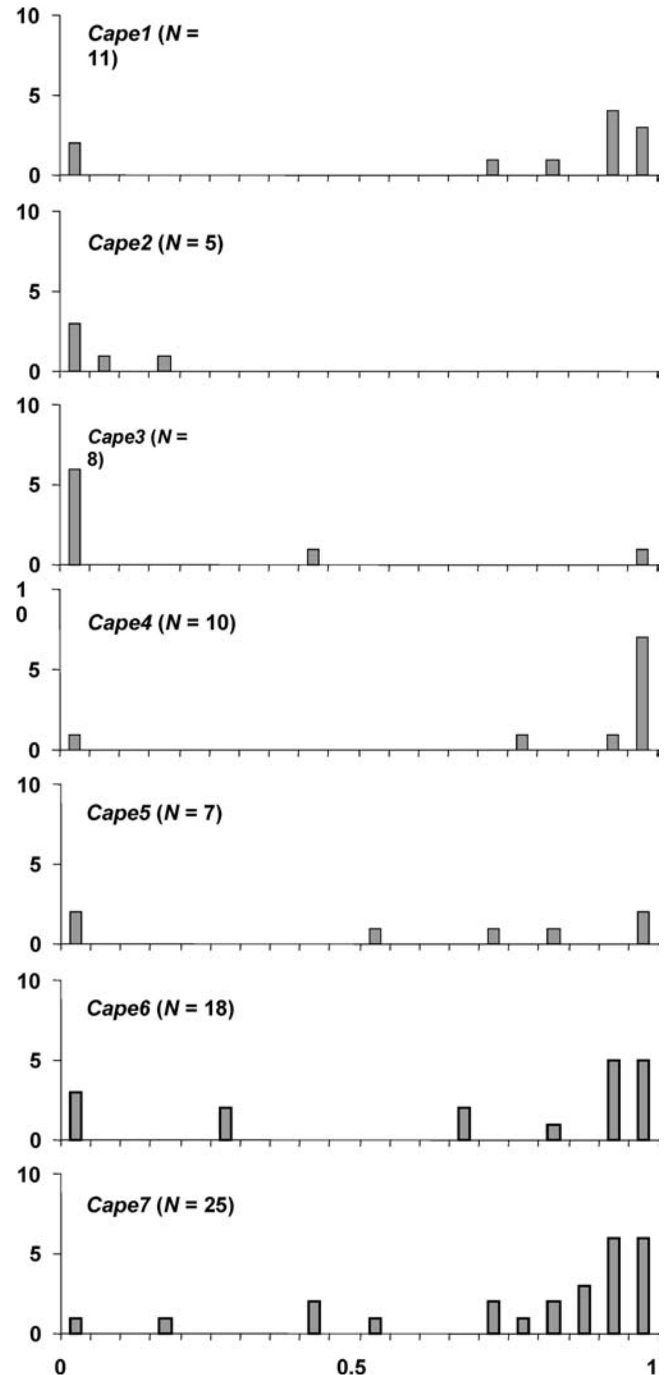

Fig. 2 *Thunnus obesus*. Distribution of individual assignments for each sample taken off southern Africa (*Cape-1* to *Cape-7*). Assignments were based on all available multilocus genotypes. Probability to belong to the Indian or Atlantic population as in the legend to Fig. 1

assignment procedure was carried out on a simulated hybrid population of size $N=84$ (i.e. sample size identical to the actual Cape sample; Fig. 1). A total of 67 individuals were assigned with intermediate *P*-values ($0.10 < P < 0.90$), and only 3 simulated hybrid individuals were assigned with $P > 0.95$ to the Indian population (vs. 23 in the actual Cape sample; Fig. 1). In this simulation, no individual was correctly assigned to the Atlantic population (vs. 18 in the real sample; Fig. 1). A Kolmogorov–Smirnov two-sample test for comparing the observed distributions of posterior probabilities (observed vs. simulated hybrid data) was significant ($P < 0.05$). These results therefore dismiss the hypothesis of hybrid origin for the Cape individuals.

Discussion

Hypothesis of two species versus secondary contact

Nucleotide divergence between the α and β mtDNA types of bigeye tuna (*Thunnus obesus*) is of the same order as the divergence between yellowfin, blackfin, and longtail tunas (Alvarado Bremer et al. 1998; Takeyama et al. 2001). The fact that such divergent clades occur in sympatry suggests they might represent two species. Here, Atlantic bigeye tuna of the two mitochondrial types (α and β) did not differ at the four nuclear loci examined. This fails to support the hypothesis that the two mitochondrial clades may correspond to two species.

A possible explanation for the presence of two highly divergent mitochondrial clades within a species is secondary contact and interbreeding between populations subsequent to their geographical isolation for a long period. The co-occurrence of similarly divergent mitochondrial clades within populations of two other migratory oceanic fishes, blue marlin (*Makaira nigricans*) and sailfish (*Istiophorus platypterus*), is thought to result from past geographic isolation followed by recent secondary contact (Graves and McDowell 2003, and references therein). Apart from a few α/clade II haplotypes found in the western part of the Indian Ocean and a single one from the eastern Pacific, all Indian and Pacific bigeye tuna samples analyzed so far (Alvarado Bremer et al. 1998; Chow et al. 2000; Appleyard et al. 2002a; present results) were of the mitochondrial type β/clade I/BET1. The similar, asymmetric patterns in the other large pelagic fish species (swordfish and blue marlin) (Graves and McDowell 2003, and references therein) suggest a common evolutionary history. Thus, secondary contact may principally have occurred in the Atlantic Ocean, following the immigration of Indo-Pacific bigeye tuna into the Atlantic Ocean, and not, or only marginally so, in the opposite direction. It is possible that this secondary contact occurred temporarily and that the Atlantic and Indo-Pacific populations presently are geographically isolated or quasi-isolated again (but see below).

Nuclear differentiation between oceans

Several large pelagic fish species show genetic differences between the Atlantic and the Indo-Pacific, with a geographic break at the tip of southern Africa. Apart from bigeye tuna (Alvarado Bremer et al. 1998; Chow et al. 2000; present results), this includes albacore (*Thunnus alalunga*, Chow and Ushiyama 1995), blue marlin (Finnerty and Block 1992; Graves and McDowell 1995; Buonaccorsi et al. 1999, 2001), swordfish (*Xiphias gladius*, Alvarado Bremer et al. 1996; Chow et al. 1997; Chow and Takeyama 2000), and sailfish (Graves and McDowell 1995). In contrast, no population partitioning between the Atlantic and Indo-Pacific Oceans has been observed in yellowfin tuna (Scoles and Graves 1993; Ward et al. 1997). The present paper, which reports on allele frequencies at both mtDNA and nDNA loci in both Atlantic and Indo-Pacific bigeye tuna, allows comparisons between markers in a global-scale analysis of population structure. MtDNA differentiation between Atlantic and Indo-Pacific Oceans was confirmed from the analysis of the *ATCO* fragment in new samples (NWA, Tob, S, S2, Mad and IBE; Table 1) in addition to those analyzed previously (CNA, Cape, Peru) [for previous results see Chow et al. (2000)]. Genetic differences were also evident at some nuclear loci between Atlantic and Indo-Pacific bigeye tuna. Allele frequencies at the second growth hormone intron (*GH2*) and, to a lesser extent, at the microsatellite locus µ208 supported the hypothesis of gene flow disruption between the Atlantic and the Indo-Pacific. Genetic data from two other, less variable loci (*Amyl* and *Anom231*) failed to support this genetic break. Overall, both nDNA and mtDNA data indicate genetic and geographic separation of the bigeye tuna populations of the two oceans. However, geographic isolation between the two populations may not need to be complete for the maintaining of allele-frequency differences, particularly if gene flow between the populations is mainly unidirectional.

Contact zone off southern Africa

Hardy–Weinberg and linkage disequilibria, as observed in the Cape samples, pointed to a Wahlund effect, suggesting that Atlantic and Indo-Pacific bigeye tuna simply mix temporarily off southern Africa. Assignment tests showed that roughly two-thirds were of Indo-Pacific origin and one-third of Atlantic origin. As noted by Alvarado Bremer et al. (1998), the westward Agulhas current may facilitate migration of tuna from the Indian Ocean to the Southeast Atlantic. It was shown that the different subsamples (Cape-1 to Cape-7) have different genetic proportions of Atlantic and Indo-Pacific individuals (Fig. 2). This result may have important management implications to further understand the dynamics and patterns of habitat use of each stock across southern Africa using genetic monitoring.

Oceanographic conditions may be more influential in determining the presence of bigeye tuna than geographic delineation. However, due to the unknown precise location of some Cape samples (Table 1), it was not possible to further explore the importance of the oceanographic conditions off southern Africa to bigeye population admixture in space and upon season.

Evidence of sex-biased dispersal?

Approximately 10-fold to 20-fold differences were found between estimates of population differentiation based on uniparentally inherited mtDNA and those based on biparentally inherited nuclear loci. Higher genetic differentiation for mtDNA is expected from the fourfold smaller effective population size of mtDNA relative to nuclear loci (Birky et al. 1983). This makes mtDNA more susceptible to the effect of genetic drift. However, based on the outcome of genetic models (Mossman and Waser 1999), such large differences are unlikely to simply reflect the differing population sizes of the two genomes, and may suggest sex-biased dispersal or sex-biased philopatry. As mtDNA has maternal-only inheritance, patterns in mtDNA differentiation reflect female-mediated dispersal processes only. If female-biased dispersal (male philopatry) occurs, mtDNA is homogeneous, but nDNA exhibits differentiation. On the contrary, if male-biased dispersal (female philopatry) occurs, mtDNA shows differentiation, but nDNA does not. A component of site fidelity is certainly present in bigeye tuna (Shaefer and Fuller 2002), but unfortunately it is not known if it preferentially affects females rather than males, or alternatively if long-distance migrants are preferentially males. Sex-biased dispersal has been reported for other marine organisms with potential for trans-oceanic dispersal, such as white and mako sharks (Pardini et al. 2001; Schrey and Heist 2003, respectively), sperm whale (Lyrholm et al. 1999), Dall's porpoise (Escorza-Treviño and Dizon 2000), and green turtles (FitzSimmons et al. 1997). In marine bony fishes, sex-biased dispersal/philopatry has been suggested in Patagonian toothfish (Appleyard et al. 2002b; Shaw et al. 2004) and in sea bass (Lemaire et al. 2005). The issue of sex-biased dispersal (or philopatry) remains controversial though, because it is difficult to reject alternative explanations. Although genetic models (Mossman and Waser 1999; Prugnolle and de Meeûs 2002) predict that sex-biased dispersal will lead to differences between nDNA and mtDNA-based estimates of genetic differentiation as observed in bigeye tuna, departure from the theoretical assumptions of the models, such as asymmetric gene flow (FitzSimmons et al. 1997), differential genetic drift among sexes, unbalanced sex ratio (as reported for bigeye; Miyabe 2003), and differences in selection and mutation rates, may have similar effects (Prugnolle and de Meeûs 2002). Also, it is not clear how far the violation of the assumption of equilibrium between mutation and drift, as is obviously the case for mtDNA in Atlantic bigeye tuna because of the deep phylogenetic subdivision between types α and β, affects the outcome of such models.

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