Preliminary study of albacore (*Thunnus alalunga*) stock differentiation inferred from microsatellite DNA analysis

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The albacore (*Thunnus alalunga*) is a highly migratory large pelagic tuna, common from tropical to temperate areas of all oceans, including the Mediterranean Sea. Although albacore populations of each ocean or each hemisphere have been managed separately, relationships between albacore populations of northern and southern hemispheres within an ocean are controversial. Differences in morphometry, movement, and catch statistics of albacore between northern and southern hemispheres within Atlantic and Pacific Oceans have been reported (Kurogane and Hiyama, 1958; Ishii, 1965; Nakamura, 1969). Examining mtDNA variation, Chow and Ushiama (1995) detected very little genetic difference between samples from northern and southern hemispheres. They proposed that minor gene flow may have occurred between albacore populations of northern and southern hemispheres—enough to prevent genetic differentiation. However, it is also possible that insufficient time has elapsed since population subdivision for mtDNA genotypic rearrangement. Because all tuna species of the genus *Thunnus* are thought to be phylogenetically new (Chow and Kishino, 1995), use of highly variable genetic markers may be necessary to investigate genetic differentiation between stocks.

Recently, Takagi et al. (1999) isolated four microsatellite loci from Pacific northern bluefin tuna (*Thunnus thynnus orientalis*) and demonstrated successful cross-species amplification of homologous microsatellites in other tuna species. In our study, we used these microsatellite primers to evaluate genetic variation within and between albacore samples from the Atlantic and the Pacific Oceans and present genetic evidence of population structuring between and within ocean samples of albacore.

Materials and methods

Five albacore samples were drawn from archival stock materials in the National Research Institute of Far Seas Fisheries laboratory. One sample each came from the Northwest Pacific (NW Pacific; Japan), Southwest Pacific (SW Pacific; Australia), Southeast Pacific (SE Pacific; Chile and Peru), and two came from the Northeast Atlantic (NE Atlantic; Biscay Bay) and the Southwest Atlantic (SW Atlantic; Brazil). These samples were derived from the same sample lots used by Chow and Ushiama (1995). Nucleotide sequences of the four primer sets, PCR amplification conditions needed to amplify the four microsatellite loci (*Ttho-1**, *-4**, *-6** and *-7**) developed for Pacific northern bluefin tuna (*T. thynnus orientalis*), and electrophoresis procedures can be found in Takagi et al. (1999). Differentiation of allele frequencies between and among samples was estimated by a fixation index (F_{ST}) with Arlequin version 1.1 (Schneider et al., 1997).

Results

Alleles observed in each locus were as follows: 9 in *Ttho-1**, 29 in *Ttho-4**, 31 in *Ttho-6**, and 18 in *Ttho-7** (Table 1). All four sets of PCR primers successfully amplified scorable microsatellite loci for all samples. Observed heterozygosity (H_0) ranged from 0.391 to 0.914 at *Ttho-1**, from 0.688 to 0.886 at *Ttho-4**, from 0.548 to 0.884 at *Ttho-6**, and from 0.857 to 1 at *Ttho-7**. We found no substantial discrepancy between observed and expected number of genotypes for any locus.

All samples except NE Atlantic shared the most common alleles for all loci, whereas the NE Atlantic sample shared the most common alleles only within *Ttho-1**. The NE Atlantic sample also did not share the second most common allele with the other samples for all loci. F_{ST} among all five

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Table 1

Allele frequency and genetic variability for four microsatelite loci surveyed for albacore used in this study.

Locus	Allele (base pairs)	NW Pacific	SW Pacific	SE Pacific	SW Atlantic	NE Atlantic
Ttho-1*	172	0.000	0.000	0.021	0.000	0.000
	174	0.000	0.016	0.000	0.000	0.000
	176	0.075	0.065	0.043	0.054	0.065
	178	0.000	0.097	0.096	0.000	0.032
	180	0.234	0.177	0.202	0.141	0.145
	182	0.521	0.452	0.436	0.685	0.516
	184	0.149	0.161	0.160	0.098	0.210
	186	0.000	0.032	0.043	0.022	0.032
	188	0.021	0.000	0.000	0.000	0.000
No. of samples		47	31	47	46	31
No. of allele		5	7	7	5	6
Effective no. of alleles[1]		2.82	3.62	3.70	1.99	2.96
Observed heterozygosity (H_o)		0.659	0.871	0.914	0.391	0.645
Expected heterozygosity (H_e)		0.645	0.724	0.730	0.498	0.662
Ttho-4*	134	0.000	0.000	0.000	0.000	0.032
	136	0.000	0.016	0.011	0.000	0.096
	138	0.011	0.016	0.033	0.011	0.065
	140	0.043	0.031	0.067	0.011	0.452
	142	0.032	0.031	0.167	0.000	0.032
	144	0.085	0.094	0.100	0.102	0.065
	146	0.383	0.313	0.178	0.261	0.048
	148	0.032	0.078	0.089	0.080	0.097
	150	0.011	0.094	0.056	0.023	0.032
	152	0.053	0.047	0.067	0.102	0.048
	154	0.096	0.031	0.044	0.046	0.032
	156	0.011	0.000	0.011	0.068	0.000
	158	0.032	0.047	0.000	0.057	0.000
	160	0.043	0.047	0.044	0.046	0.000
	162	0.021	0.031	0.033	0.034	0.000
	164	0.011	0.078	0.044	0.023	0.000
	166	0.053	0.000	0.000	0.023	0.000
	168	0.021	0.031	0.011	0.011	0.000
	170	0.000	0.000	0.022	0.011	0.000
	174	0.000	0.000	0.011	0.023	0.000
	176	0.000	0.000	0.000	0.023	0.000
	178	0.000	0.000	0.011	0.023	0.000
	180	0.032	0.000	0.000	0.000	0.000
	182	0.011	0.000	0.000	0.000	0.000
	184	0.011	0.000	0.000	0.000	0.000
	186	0.000	0.016	0.000	0.000	0.000
	190	0.011	0.000	0.000	0.000	0.000
	202	0.000	0.000	0.000	0.011	0.000
	232	0.000	0.000	0.000	0.011	0.000
No. of samples		47	32	45	44	31
No. of allele		20	16	18	21	11
Effective no. of alleles[1]		5.62	7.14	10.20	8.85	4.17
Observed heterozygosity (H_o)		0.851	0.688	0.844	0.886	0.710
Expected heterozygosity (H_e)		0.822	0.860	0.902	0.887	0.760
Ttho-6*	127	0.000	0.000	0.000	0.012	0.000
	129	0.000	0.000	0.000	0.012	0.000
	131	0.011	0.032	0.000	0.000	0.000
	133	0.011	0.000	0.000	0.000	0.000
	135	0.033	0.113	0.047	0.093	0.000

continued

Table 1 (continued)

Locus	Allele (base pairs)	NW Pacific	SW Pacific	SE Pacific	SW Atlantic	NE Atlantic
Ttho-6* (continued)	137	0.100	0.000	0.023	0.105	0.000
	139	0.056	0.016	0.093	0.047	0.000
	141	0.478	0.629	0.233	0.349	0.083
	143	0.067	0.048	0.081	0.105	0.017
	145	0.167	0.113	0.163	0.128	0.050
	147	0.067	0.048	0.093	0.058	0.300
	149	0.000	0.000	0.081	0.047	0.083
	151	0.011	0.000	0.023	0.012	0.017
	153	0.000	0.000	0.012	0.000	0.033
	155	0.000	0.000	0.035	0.000	0.050
	157	0.000	0.000	0.000	0.012	0.033
	159	0.000	0.000	0.000	0.023	0.050
	161	0.000	0.000	0.012	0.000	0.083
	163	0.000	0.000	0.023	0.000	0.000
	165	0.000	0.000	0.000	0.000	0.033
	167	0.000	0.000	0.023	0.000	0.050
	169	0.000	0.000	0.000	0.000	0.033
	171	0.000	0.000	0.012	0.000	0.017
	177	0.000	0.000	0.012	0.000	0.000
	183	0.000	0.000	0.000	0.000	0.017
	185	0.000	0.000	0.000	0.000	0.017
	187	0.000	0.000	0.012	0.000	0.000
	189	0.000	0.000	0.012	0.000	0.000
	191	0.000	0.000	0.012	0.000	0.000
	199	0.000	0.000	0.000	0.000	0.017
	201	0.000	0.000	0.000	0.000	0.017
No. of samples		45	31	43	43	30
No. of allele		10	7	19	13	19
Effective no. of alleles[1]		3.58	2.34	8.47	5.65	7.87
Observed heterozygosity (H_o)		0.689	0.548	0.884	0.884	0.867
Expected heterozygosity (H_e)		0.721	0.573	0.882	0.823	0.873
Ttho-7*	182	0.000	0.031	0.000	0.000	0.000
	188	0.083	0.109	0.032	0.000	0.017
	190	0.024	0.000	0.021	0.010	0.033
	192	0.024	0.063	0.075	0.052	0.000
	194	0.321	0.203	0.287	0.250	0.217
	196	0.012	0.063	0.096	0.146	0.117
	198	0.024	0.078	0.053	0.125	0.317
	200	0.083	0.172	0.074	0.135	0.050
	202	0.012	0.031	0.053	0.042	0.083
	204	0.024	0.000	0.011	0.031	0.017
	206	0.012	0.016	0.043	0.000	0.017
	208	0.060	0.078	0.032	0.031	0.050
	210	0.131	0.063	0.075	0.042	0.017
	212	0.107	0.000	0.021	0.063	0.067
	214	0.024	0.031	0.053	0.031	0.000
	216	0.024	0.031	0.043	0.010	0.000
	218	0.024	0.031	0.032	0.010	0.000
	224	0.000	0.000	0.000	0.010	0.000
No. of samples		42	32	47	48	30
No. of allele		16	14	16	15	12
Effective no. of allele		6.49	8.93	8.06	7.63	5.59
Observed heterozygosity (H_o)		0.857	0.906	0.872	0.896	1.000
Expected heterozygosity (H_e)		0.846	0.888	0.876	0.869	0.821

[1] Effective number of alleles was calculated with the equation $1/(1-H_e)$.

samples deviated considerably from zero (F_{ST}=0.018 to 0.070, P<0.001) for all loci. Significant departures of F_{ST} from zero were observed for three loci (*Ttho-1**, *Ttho-4**, and *Ttho-6**) among all samples except the NE Atlantic sample (F_{ST} =0.013 to 0.085, P<0.005) and among the three Pacific samples (F_{ST}=0.018 to 0.074, P<0.001). There were also significant differences in allele frequencies between some pairwise comparisons (Table 2). At *Ttho-1**, the SW Pacific sample showed significant difference from all other samples (all P<0.001), and there was also a significant difference between the SE Pacific and SW Atlantic samples (P=0.003). The NE Atlantic sample was significantly heterogeneous in comparison with all other samples in *Ttho-4** (all P<0.001). Difference between the NW and SE Pacific samples was also significant in this locus (P=0.001). In *Ttho-6** the NE Atlantic sample was again significantly heterogeneous in comparison with all other samples (all P<0.001). Furthermore, there were also significant differences between the NW and SE Pacific, between the SW and SE Pacific, and between the SW Pacific and SW Atlantic samples in this locus (all P<0.001). In *Ttho-7** the NE Atlantic sample showed significant differences from all three Pacific samples (P<0.001 for the NW and SW Pacific, P=0.003 for the SE Pacific) but not from the SW Atlantic sample (P=0.017).

Table 2

Pairwise comparison of F_{ST} between five albacore samples.

	NW Pacific	SW Pacific	SE Pacific	SW Atlantic
Ttho-1*				
SW Pacific	0.1401			
SE Pacific	0.005	0.099[1]		
SW Atlantic	0.018	0.249[1]	0.0461	
NE Atlantic	–0.004	0.154[1]	–0.003	0.023
Ttho-4*				
SW Pacific	0.003			
SE Pacific	0.032[1]	0.012		
SW Atlantic	0.006	–0.001	0.017	
NE Atlantic	0.151[1]	0.131[1]	0.094[1]	0.131[1]
Ttho-6*				
SW Pacific	0.020			
SE Pacific	0.038[1]	0.100[1]		
SW Atlantic	0.006	0.052[1]	0.010	
NE Atlantic	0.135[1]	0.208[1]	0.0431	0.086[1]
Ttho-7*				
SW Pacific	0.012			
SE Pacific	0.006	0.003		
SW Atlantic	0.020	0.006	0.001	
NE Atlantic	0.592[1]	0.042[1]	0.040[1]	0.017

[1] Significant difference (P<0.005).

Discussion

Because the number of alleles observed in microsatellite loci is usually large and the frequency of each allele may be low, a large sample size is necessary for satisfying subsequent statistic analyses. Ruzzante (1997) showed that a sample size of 50 <n< 100 individuals is generally satisfactory, although this size depends on allele number and frequency. Size of our samples ranged from 32 to 48, close to the lower margin of this threshold. Nevertheless, the distinct status of the Northeast Atlantic albacore sample from others is obvious. Although our study shared the same sample lots with Chow and Ushiama (1995), their mtDNA analysis revealed much less genetic differentiation between samples from the North and South Atlantic. MtDNA analysis is thought to be a more effective indicator for population subdivision than nuclear DNA (Nei and Li, 1979). But for albacore in the Atlantic, this is obviously not the case. No selection toward microsatellite alleles is obvious; therefore, differences in evolutionary rate between mtDNA and nDNA may explain differences in allele frequency distribution in the Atlantic albacore. Because mtDNA variation observed by Chow and Ushiama (1995) was much lower than the microsatellite DNA variation observed in our present study, there might have been insufficient elapsed time for unique mitochondrial genotypes to have arisen within the existing stocks. Thus, the rapidly evolving microsatellites appear to reflect albacore population subdivision.

MtDNA analyses have also failed to detect genetic difference between samples from northern and southern hemispheres within the Pacific (Chow and Ushiama, 1995). In contrast, present microsatellite analysis detected significant differences among three Pacific samples, clearly indicating that microsatellites appear to be more sensitive and powerful in detecting more subtle signals of genetic differentiation in albacore samples than mtDNA analysis. These results support several ecological and morphometric studies (Nakamura, 1969; Lewis, 1990) which assumed negligible migration of albacore across the equator in the Pacific. Separate North and South Pacific albacore stocks is a reasonable assumption because two major spawning grounds confined in the western to mid tropical Pacific are spatiotemporarily separated (Nishikawa et al., 1985). However, it is difficult to explain genetic differentiation between southwest and southeast Pacific albacore samples because no major spawning ground of albacore has been determined in the southeast Pacific. Microsatellite analysis of a sample from a different year class and a larger sample size is necessary to better define the observed genetic differences among the Pacific samples and the South Atlantic sample.

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