Global population structure of yellowfin tuna, *Thunnus albacares*, inferred from allozyme and mitochondrial DNA variation

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Abstract.—Yellowfin tuna, *Thunnus albacares*, were sampled from one region of the Atlantic Ocean, two regions of the Indian Ocean, and six regions of the Pacific Ocean. One of the Indian Ocean collections could not be allozymically analyzed; the remaining eight collections were examined for four polymorphic allozyme loci (*ADA**, *FH**, *GPI-A**, and *GPI-B**, n=540 to 677). All nine collections were examined for mitochondrial DNA variation (n=767), with two restriction enzymes (*Bcl* I and *Eco* RI) that detect polymorphic restriction sites in yellowfin tuna. Allele frequencies at three of the allozyme loci were homogeneous across collections, whereas *GPI-A** showed highly significant differentiation (P<0.001). The *GPI-A** data, taken together with the geographic location of the collections, suggested the existence of at least four yellowfin tuna stocks: Atlantic Ocean, Indian Ocean, west-central Pacific Ocean, and east Pacific Ocean. Mitochondrial DNA differentiation was more limited, but spatial heterogeneity of the 24 observed haplotypes over the nine regions (P=0.048) and three oceans (P=0.009) was significant. The mtDNA data did not differentiate west-central Pacific Ocean collections from east Pacific Ocean collections but did support the separation of Atlantic Ocean, Indian Ocean, and Pacific Ocean stocks.

Manuscript accepted 10 February 1997.
Fishery Bulletin 95:566–575 (1997).

The yellowfin tuna, *Thunnus albacares* (Bonnaterre), supports important fisheries in tropical and subtropical oceans. Catches have increased from about 600,000 metric tons (t) in 1982 and 1983 to about 1,100,000 t in 1993 and 1994; in 1994, about 63% of the catch came from the Pacific Ocean, about 24% from the Indian Ocean, and 14% from the Atlantic Ocean (FAO, 1996). Given the size and circumglobal nature of the resource, there is considerable management interest in determining stock structures.

It is only comparatively recently that yellowfin tuna has been recognized as a single species (Gibbs and Collette, 1967); its high degree of morphological variation led Jordan and Evermann (1926) to recognize seven yellowfin tuna species. However, a major morphometric study by Royce (1964) revealed that intra-oceanic differences could be greater than interoceanic differences and that several characters showed clinal variation. He concluded that the morphometric data are best explained by a single worldwide pantropical species, a conclusion confirmed by Gibbs and Collette (1967).

Most stock structure studies of yellowfin tuna have focused on the large Pacific Ocean component of the catch. Here, tagging experiments indicated that yellowfin tuna usually migrate hundreds rather than thousands of kilometers and that their movements do not range far both east-west or north-south (Joseph et al., 1964; Bayliff, 1979; Hunter et al., 1986; Lewis, 1992). Morphometric studies have provided commensurate results, with Mexico and Ecuador fish being much more similar to one another than to fish from the central (Hawaii) and western (Australia, Japan) Pacific (Schaefer, 1991). Studies of the microchemical composition of larval portions of otoliths in West Pacific fish (Indonesia, Philippines, Coral Sea, Hawaii) have shown some differences, indicating that such analyses may be useful in determination of spawning origins (Gunn and Ward[1]; Gunn[2]). Genetic studies of four to five polymorphic

[1] Gunn, J. S., and R. D. Ward. 1994. The discrimination of yellowfin tuna sub-populations within the AFZ. Phase 1: a pilot study to determine the extent of genetic and otolith microchemical variability in populations from different parts of the Pacific and Indian Oceans. Final Report (91/27) to Fisheries Research and Development Corporation, Deakin, ACT, Australia.

[2] Gunn, J. S. 1996. CSIRO Division of Marine Research, Hobart, Tasmania, Australia. Unpubl. data.

allozyme loci in Pacific Ocean collections have shown significant spatial heterogeneity at one locus (*GPI-A**); the common allele in western and central regions differed from that in the east (Sharp, 1978; Ward et al., 1994). This finding either indicates the existence of two reproductively isolated groups of yellowfin tuna in the Pacific Ocean or suggests that selection pressures are different in the two regions. There is no evidence of mitochondrial DNA (mtDNA) differentiation between eastern Pacific and western Pacific yellowfin tuna (Scoles and Graves, 1993; Ward et al., 1994).

In the Atlantic Ocean, where it was once assumed that there were separate eastern and western stocks, recent taggings of large yellowfin tuna have resulted in 15 trans-Atlantic recoveries (ICCAT, 1992b); a single stock is now assumed (ICCAT, 1995). There have been no genetic comparisons of eastern and western Atlantic yellowfin tuna.

The extent of genetic differentiation of yellowfin tuna from different oceans has been little studied. Suzuki (1962) found no differences in the incidence of the Tg2 blood group antigen in fish from the equatorial Pacific and Indian Oceans. Scoles and Graves (1993) found no significant differentiation in mtDNA from one west Atlantic collection and five Pacific collections (each of 20 fish). Here we compare genetic variation in collections from the Pacific, Indian, and Atlantic oceans. We used larger sample sizes than those used in the study by Scoles and Graves (1993) and examined both allozyme and mtDNA variation to see if the increased statistical power would enable us to reject the null hypothesis of no interoceanic genetic differentiation.

Materials and methods

Samples were collected from one region of the Atlantic Ocean, two regions of the Indian Ocean, and six regions of the Pacific Ocean. Details of most of the Pacific collections (Philippines, Coral Sea, Kiribati, Hawaii, California, and Mexico) are given in Ward et al. (1994). For the present paper, the 1991 and 1992 Hawaii collections were pooled. A second Philippines collection was collected in October–December 1994. This showed no significant genetic differentiation from the earlier collection; therefore the two collections were pooled for our study. The Atlantic collection was taken from the Caribbean Sea (Gulf of Mexico, approx. 28°N, 88°W) in September 1993. The Indian Ocean collections were taken from off Sri Lanka (approx. 6°N, 80°E) and off the Seychelles (approx. 7°S, 52°E) in December 1994. White muscle samples were flown (airfreight) frozen to Hobart and stored at –80°C.

Specimens were studied by allozyme and mtDNA analysis. The experimental methods are given in Ward et al. (1994). Four allozyme loci known to be polymorphic in white muscle were examined: *ADA** (adenosine deaminase, EC 3.5.4.4), *FH** (fumarate hydratase, EC 4.2.1.2), *GPI-A**, and *GPI-B** (glucose-6-phosphate isomerase, EC 5.3.1.9). MtDNA variation was examined by using two restriction enzymes (*Bcl* I and *Eco* RI) known to discriminate most of the mtDNA haplotypes revealed by eight restriction enzymes in an earlier survey (*Bam* HI, *Ban* I, *Bcl* I, *Eco* RI, *Hind* III, *Pvu* II, *Sal* I, and *Xho* I—see Ward et al., 1994).

The homogeneity of allele and haplotype frequencies of the collections was tested by the randomized Monte Carlo chi-square procedure of Roff and Bentzen (1989). For each test, 2,000 randomizations of the data were carried out, each giving a randomized chi-square value (χ^2_{null}). The probability that the null hypothesis of genetic homogeneity was correct was given by $P = n/2{,}000$, where n is the number of randomizations that generate $\chi^2_{null} \geq \chi^2$ and where χ^2 is the chi-square value given by the actual observations.

The extent of genetic differentiation among collections was quantified by Nei's gene diversity statistic G_{ST} (Nei, 1987), which estimates the proportion of total genetic variation attributable to differentiation between populations. For each allozyme locus, G_{ST} was estimated as $(H_T - H_S)/H_T$, where H_T represents total heterozygosity and H_S is average (Hardy-Weinberg expected) population heterozygosity. The mtDNA data were analyzed in a similar way, treating haplotypes as alleles and H_T and H_S as diversity estimates. The proportion or magnitude of G_{ST} generated by sampling error, which we have termed $G_{ST.null}$, was estimated with a bootstrapping program, with the observed allele or haplotype frequencies and collection sizes. Simulations were run 1,000 times to provide a mean value of $G_{ST.null}$ and a standard deviation. The probability of obtaining a value of $G_{ST.null}$ as large or larger than that obtained from the actual observations of G_{ST} was given by $P = n/1{,}000$, where n is the number of randomizations that generate $G_{ST.null} \geq G_{ST}$. Values of P less than 0.05 indicated significant differentiation between areas that could not be explained by sampling error alone.

Bonferroni adjustments of significance levels, to correct for multiple tests, were carried out with the sequential procedure advocated by Hochberg (1988). Tests are ordered according to their probability value. The highest probability value, P_m, is compared with the significance value α. Here we initially set $\alpha = 0.05$. If $P_m \geq \alpha$, that test is judged to be nonsignificant, and comparisons continue with subsequent probabilities, each compared with a modified signifi-

cance level = $\alpha/(1+i)$, where i is the number of tests already performed. When a test is significant, it and all subsequent tests are deemed significant.

Cluster analysis of the allozyme allele frequency data and the mtDNA haplotype frequency data used the UPGMA (unweighted pair-group method using averages) algorithm with Nei's (1978) unbiased genetic distance measure, as implemented in BIOSYS-1 (Swofford and Selander, 1981).

Estimates of mtDNA nucleotide sequence diversity and divergence (Nei and Tajima, 1981; Nei, 1987) were made with REAP vers.4.0 (see McElroy et al., 1992), and population divergences were clustered by using UPGMA.

Results

The Seychelles muscle samples were partially degraded on arrival, and could not be confidently screened for allozyme determinations, although mtDNA analysis presented no problems. Because it is sometimes difficult to distinguish tuna species, we usually find a small percentage (3–5%) of non-yellowfin tunas among nominal yellowfin tuna collections. These misidentified fish can be recognized by aberrant allozyme (Graves et al., 1988; Elliott and Ward, 1995) and mtDNA patterns (Grewe[3]). For example, five (3.7%) of the 135 Philippines samples collected in 1994 proved to be bigeye tuna, *Thunnus obesus*. However, at times, the proportion of misidentified fish can be much higher: 18 (46.2%) of the 39 "yellowfin tuna" from Sri Lanka proved to be bigeye tuna. The misidentified fish were excluded from the following analyses.

Allozyme allele frequencies at four polymorphic loci (*ADA**, *FH**, *GPI-A**, *GPI-B**) for eight collections (Table 1) and mtDNA haplotype frequencies for nine collections (Table 2) were determined.

No significant deviations from Hardy-Weinberg expectations were recorded for any allozyme locus. Heterogeneity chi-square analyses (Table 3) of allele frequencies revealed no significant differentiation for three loci (*ADA**, *FH**, and *GPI-B**), but highly significant heterogeneity at the fourth locus, *GPI-A** ($P<0.001$, $\alpha=0.0125$). Genetic diversity (G_{ST}) analyses (Table 3) indicated that for *ADA**, *FH**, and *GPI-B**,

[3] Grewe, P. M. 1993. CSIRO Division of Marine Research, Hobart, Tasmania, Australia. Unpubl. data.

Table 1

Allozyme allele frequencies and sample sizes (n). GOM = Gulf of Mexico, S.Lan. = Sri Lanka, Philipp. = Philippines, Cl. Sea = Coral Sea, Calif. = California.

		Atlantic	Indian	Pacific					
Locus	Allele	GOM	S. Lan.	Philipp.	Cl. Sea	Kiribati	Hawaii	Calif.	Mexico
*ADA**	125	0.005	—	0.003	—	—	—	—	—
	115	0.414	0.310	0.330	0.306	0.399	0.361	0.317	0.359
	100	0.548	0.643	0.622	0.638	0.567	0.609	0.671	0.628
	85	0.033	0.048	0.045	0.056	0.034	0.030	0.012	0.013
	n	105	21	176	98	89	115	41	39
*FH**	130	0.118	0.091	0.081	0.117	0.086	0.076	0.051	0.075
	100	0.875	0.909	0.910	0.878	0.900	0.920	0.949	0.925
	75	0.007	—	0.009	0.005	0.014	0.004	—	—
	n	68	11	111	98	70	112	39	29
*GPI-B**	–20	0.015	—	—	—	—	0.004	—	—
	–60	0.180	0.167	0.176	0.163	0.233	0.113	0.187	0.231
	–100	0.806	0.833	0.824	0.837	0.767	0.878	0.813	0.769
	–125	—	—	—	—	—	0.004	—	—
	n	103	21	176	98	88	115	40	39
*GPI-A**	145	—	—	0.003	—	—	—	—	—
	135	0.045	—	0.015	0.036	0.011	0.035	0.122	0.077
	100	0.624	0.286	0.651	0.683	0.673	0.609	0.305	0.231
	75	0.332	0.714	0.328	0.281	0.316	0.357	0.573	0.692
	40	—	—	0.003	—	—	—	—	—
	n	101	21	175	98	87	115	41	39

slightly less than 1% of the observed diversity arose from differences between collections and could be attributed to sampling error alone ($G_{ST.null}$). For *GPI-A**, the observed value of G_{ST}, at 12%, was much larger than the value attributable to sampling error (about 1%). The "true" G_{ST} estimate of *GPI-A** —the difference between G_{ST} and $G_{ST.null}$ —is thus around 11%, indicating that about 11% of the observed diversity at the *GPI-A** locus comes from differences between collections.

The *GPI-A** heterogeneity (Fig. 1) was further explored by comparing all collections pairwise (Fig. 2; Table 4). This comparison essentially revealed two groups of collections: 1) the west-central Pacific Ocean and the Atlantic Ocean (Gulf of Mexico) collections; and 2) the Indian Ocean (Sri Lankan) and eastern Pacific Ocean (Californian and Mexican) collections. Within each of these two groups there was no significant differentiation, but between them differentiation was marked. This conclusion holds after Bonferroni corrections of α levels for multiple tests. The *GPI-A**100 allele was the most frequent allele in the west-central Pacific Ocean and the Atlantic Ocean group, whereas the *GPI-A**75 allele was the more frequent allele in the Indian Ocean and the eastern Pacific Ocean group. The genetic differentiation of the Atlantic Ocean collection from the Indian Ocean collection suggests that fish from these areas constitute separate stocks; the separation of the Indian Ocean collection from the west-central Pacific Ocean collections suggests that fish from these areas constitute separate stocks; the separation of the west-central Pacific Ocean collections from the eastern Pacific Ocean collections suggests that fish from these areas constitute separate stocks; and the separation of the eastern Pacific Ocean collections

Table 2

Mitochondrial DNA haplotype frequencies (*Bcl* I and *Eco* RI haplotypes respectively), sample sizes (n), haplotype diversities (h) and percent nucleotide diversities (% n.d.). Abbreviations are defined in Table 1. Seych. = Seychelles.

		Atlantic	Indian		Pacific					
Locus	Haplotype	GOM	Seych.	S. Lan.	Philipp.	Cl. Sea	Kiribati	Hawaii	Calif.	Mexico
mtDNA	AA	0.266	0.319	0.381	0.286	0.340	0.443	0.276	0.294	0.325
	AB	0.543	0.407	0.333	0.509	0.402	0.364	0.537	0.463	0.425
	AC	—	—	—	—	—	0.011	0.015	—	—
	AF	—	0.011	—	—	—	—	0.007	0.049	0.025
	AG	—	—	—	0.006	—	—	0.007	—	—
	BA	0.011	0.011	—	0.025	0.010	—	0.007	0.049	—
	BB	0.064	0.066	—	0.031	0.052	0.068	0.060	0.073	0.025
	CA	0.032	0.011	—	0.031	0.062	0.011	0.015	0.024	0.050
	CB	0.021	0.088	0.286	0.068	0.103	0.057	0.045	0.024	0.125
	CO	0.021	—	—	—	—	—	—	—	—
	DB	—	—	—	—	0.010	—	—	—	—
	EB	—	—	—	—	0.021	—	—	—	—
	LB	—	—	—	—	—	0.011	—	—	—
	MB	—	—	—	—	—	0.011	—	—	—
	NB	—	—	—	0.019	—	—	0.007	—	—
	PB	—	—	—	—	—	—	0.015	0.024	—
	OA	—	—	—	0.006	—	—	—	—	0.025
	OB	—	—	—	0.006	—	0.023	—	—	—
	QA	—	0.011	—	—	—	—	—	—	—
	QB	0.011	0.011	—	—	—	—	0.007	—	—
	WA	0.032	0.033	—	—	—	—	—	—	—
	ZB	—	0.022	—	0.006	—	—	—	—	—
	A2B	—	—	—	0.006	—	—	—	—	—
	Q2B	—	0.011	—	—	—	—	—	—	—
	n	94	91	21	161	97	88	134	41	40
	h	0.634	0.727	0.695	0.655	0.712	0.670	0.633	0.705	0.712
	% n.d.	0.998	1.263	1.017	1.017	1.174	1.027	0.901	1.099	1.047

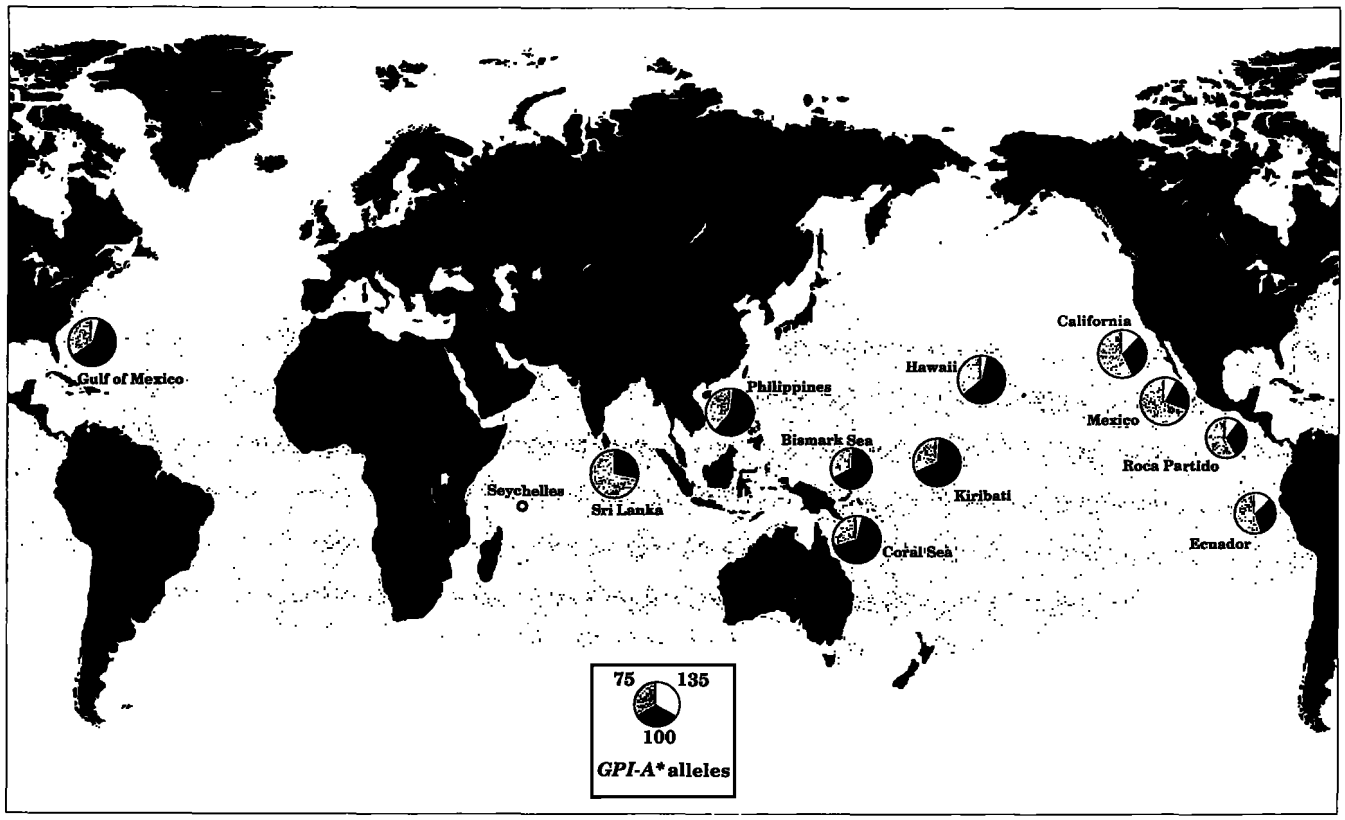

Figure 1

Map of sample sites showing *GPI-A** gene frequencies in yellowfin tuna, *Thunnus albacares*. Larger circles represent our data (Table 1), the three smaller circles (Bismark Sea, Roca Partido, and Ecuador) data are from Sharp (1978). The location of the Seychelles sample, examined for mtDNA variation but not for *GPI-A** variation, is identified. The shaded area represents the approximate global distribution of yellowfin tuna.

from the Atlantic Ocean collection suggests that these fish constitute separate stocks. Thus the *GPI-A** data, taken together with the spatial orientation of these collections, indicate the existence of at least four yellowfin tuna stocks: Atlantic Ocean, Indian Ocean, west-central Pacific Ocean, and east Pacific Ocean.

Six of the 24 mtDNA haplotypes (CO, QA, WA, ZB, A2B, Q2B, see Table 2) were not recorded in the earlier survey of Ward et al. (1994) but were rare (frequencies less than 3.5%). Fragment sizes for most haplotypes are given in Ward et al. (1994), but a full list is available on request. Haplotype (nucleon) diversities per collection ranged from 0.633 to 0.727 (mean estimate of 0.683) (Table 2). Percent nucleotide diversities per collection ranged from 0.998 to 1.263 (mean estimate of 1.061) (Table 2).

Table 3

Analyses of genetic differentiation among the samples.

Locus	Number of fish	Number of alleles/haplotypes	Heterogeneity χ^2 analysis		Genetic diversity analysis		
			χ^2	P	G_{ST}	$G_{ST.null}$±SD	P
*ADA**	684	4	17.326	0.666	0.006	0.008 ±0.004	0.569
*FH**	538	3	9.241	0.821	0.006	0.011 ±0.008	0.736
*GPI-B**	680	4	29.256	0.128	0.008	0.008 ±0.005	0.370
*GPI-A**	677	5	131.416	<0.001	0.118	0.008 ±0.004	<0.001
mtDNA	767	24	227.743	0.048	0.023	0.015 ±0.005	0.071

Table 4

Pairwise comparisons of *GPI-A** allele frequencies (*P* above, chi square below). GOM = Gulf of Mexico.

	GOM	Sri Lanka	Philippines	Coral Sea	Kiribati	Hawaii	California	Mexico
GOM	—	<0.001	0.156	0.468	0.129	0.769	<0.001	<0.001
Sri Lanka	21.700	—	0.001	<0.001	<0.001	0.001	0.031	0.214
Philippines	5.938	24.115	—	0.264	0.965	0.291	<0.001	<0.001
Coral Sea	1.586	28.644	4.870	—	0.270	0.237	<0.001	<0.001
Kiribati	3.905	22.586	1.196	2.630	—	0.203	<0.001	<0.001
Hawaii	0.493	19.130	4.635	2.824	3.280	—	<0.001	<0.001
California	24.850	6.047	46.871	35.021	37.324	25.374	—	0.289
Mexico	34.934	3.579	51.193	46.401	44.495	33.366	2.526	—

Table 5

Pairwise comparisons of mtDNA haplotype frequencies (*P* above, chi square below). GOM = Gulf of Mexico.

	GOM	Seychelles	Sri Lanka	Philippines	Coral Sea	Kiribati	Hawaii	California	Mexico
GOM	—	0.277	0.009	0.089	0.025	0.009	0.384	0.285	0.076
Seychelles	14.079	—	0.549	0.062	0.188	0.212	0.200	0.478	0.707
Sri Lanka	23.110	9.697	—	0.269	0.369	0.171	0.111	0.025	0.612
Philippines	19.823	22.529	14.013	—	0.223	0.025	0.428	0.207	0.597
Coral Sea	18.085	16.020	7.785	15.432	—	0.087	0.053	0.092	0.683
Kiribati	21.523	17.480	11.862	22.368	15.117	—	0.086	0.093	0.253
Hawaii	13.801	18.574	18.760	15.533	19.300	18.754	—	0.667	0.378
California	11.888	11.682	13.905	17.165	14.055	16.316	8.888	—	0.383
Mexico	15.622	9.718	5.017	10.188	7.203	12.214	13.080	8.473	—

A chi-square test (Table 3) showed that the mtDNA haplotype variation across all nine regions was just significant (α=0.05, P=0.048, with the standard 2,000 replicates, and P=0.045, with 10,000 replicates). Genetic diversity analysis (Table 3) gave a result bordering on significance (P=0.071, with a "true" G_{ST} of about 1%). All collections were compared pairwise with chi-square tests (Table 5) to determine which collections contributed most to the marginal chi-square differentiation. Although some pairs appeared significantly different (e.g. Gulf of Mexico versus Sri Lanka, P=0.009; Gulf of Mexico versus Kiribati, P=0.009), none was significant after Bonferroni adjustments for table-wide comparisons.

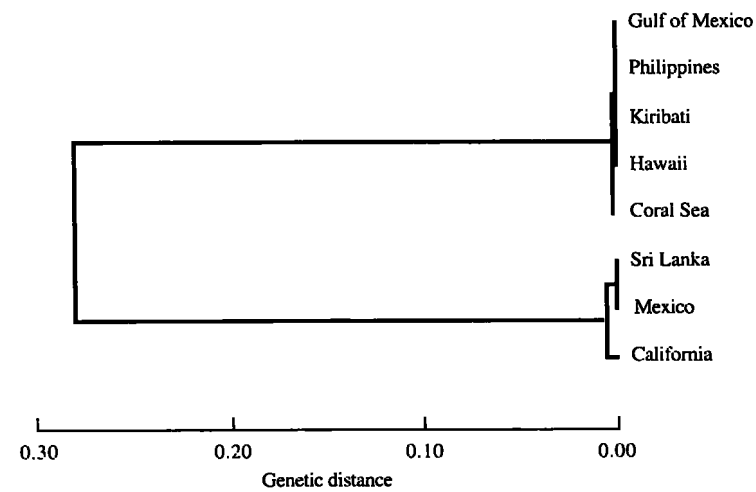

Figure 2

UPGMA phenogram for the *GPI-A** locus constructed from Nei's (1978) unbiased genetic distance.

Two UPGMA dendrograms were estimated. One, based on mtDNA haplotype frequencies alone (Fig. 3A), showed the maximal genetic-distance estimates among collections to be about 0.05 — much less than the major *GPI-A** split of nearly 0.30 (Fig. 2). The second, based on percent sequence divergence (Fig. 3B), confirmed the high degree of similarity among the collections. After correcting for within-collection nucle-

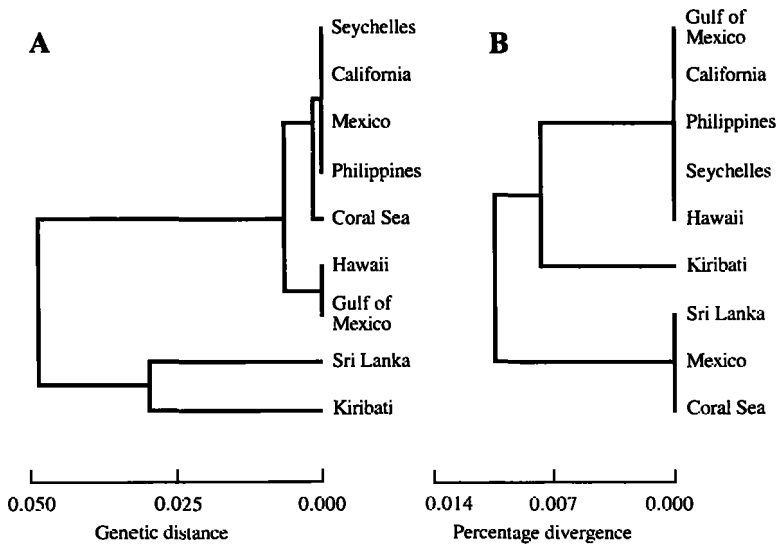

Figure 3

UPGMA phenograms for the mtDNA data with (**A**) unbiased genetic distance (Nei 1978), and (**B**) percentage nucleotide divergence (Nei, 1987).

otide divergence, pairwise nucleotide divergence ranged from 0.040% to –0.025% (mean 0.004%). There was little correspondence between these two mtDNA dendrograms, and this lack of correspondence, together with the low distances observed, suggests that the tree topologies are unreliable.

Because there is no significant mtDNA differentiation between the six Pacific Ocean collections (Table 5; and Ward et al., 1994) nor between the two Indian Ocean collections (Table 5), the collections within each ocean were pooled to test for interoceanic differences. A comparison of the three oceans yielded a chi-square analysis that was significant (P=0.009, α=0.05) and a genetic diversity analysis bordering on significance (observed G_{ST}=0.010, $G_{ST:null}$ =0.005 ±0.003, P=0.059). A pairwise comparison of the oceans showed that all pairs were significant (Indian versus Atlantic, P=0.047, α=0.05; Pacific versus Atlantic, P=0.017; Pacific versus Indian, P=0.009).

Finally, the mtDNA data were analyzed to see whether they offered any support to the conclusion from the *GPI-A** data that there are (at least) four yellowfin tuna stocks. The four putative stocks consisted of the following units: Atlantic (Gulf of Mexico), Indian (Seychelles and Sri Lanka), west-central Pacific (Coral Sea, Kiribati, Philippines, Hawaii), and east Pacific (California and Mexico). Chi-square analysis of mtDNA data from these four regions indicated limited but significant (P=0.024) heterogeneity. Although none of the six pairwise comparisons was significant following Bonferroni correction to α levels (west-central Pacific versus east Pacific, P=0.456, α=0.05; Indian versus east Pacific, P=0.316, α=0.025; Atlantic versus east Pacific, P=0.119, α=0.017; Atlantic versus Indian, P=0.047, α=0.0125; Atlantic versus west-central Pacific, P=0.032, α=0.010; Indian versus west-central Pacific, P=0.0135, α=0.008), the three pairwise comparisons of the Atlantic Ocean, Indian Ocean, and west-central Pacific all showed P-values less than 0.05.

Clearly, the mtDNA data do not differentiate west-central Pacific Ocean collections from east Pacific Ocean collections but, considering the interocean analyses alone, do provide some support for the delineation of Atlantic Ocean, Indian Ocean, and Pacific Ocean stocks.

Discussion

Samples of yellowfin tuna from the Pacific, Indian, and Atlantic oceans were compared with respect to four polymorphic allozyme loci and with respect to mtDNA variants.

No significant allele frequency differences were observed for three of the allozyme loci, but the fourth locus, *GPI-A**, showed considerable differentiation. Across all collections, the "true" G_{ST} indicated that about 11% of the variation at this locus was attributable to differences between collections. Two genetically distinguishable groups were apparent. One consists of eastern Pacific Ocean and Indian Ocean fish, with a high frequency of the *GPI-A**75 allele, the other of Atlantic Ocean and west-central Pacific Ocean fish, with a high frequency of the *GPI-A**100 allele. Because there are no migration routes between the eastern Pacific Ocean (California and Mexico) and the Indian Ocean that avoid the west-central Pacific Ocean, and between the Atlantic Ocean and west-central Pacific Ocean that avoid the Indian Ocean, there is reason to believe that there are at least four stocks of yellowfin tuna: Atlantic Ocean, Indian Ocean, west-central Pacific Ocean, and eastern Pacific Ocean.

Sharp (1978) also examined *GPI-A** allele frequencies in western and eastern Pacific populations. His *GPI-A** allele frequencies for collections from Ecuador and Mexico were very similar to our California and Mexico frequencies, and his *GPI-A** frequencies

from the Bismarck Sea in the western Pacific were very similar to our western Pacific Ocean frequencies (Ward et al., 1994), supporting the separation of western and eastern Pacific stocks. Another allozyme study (Fujino, 1970) failed to find differences between Hawaiian and eastern Pacific fish for an esterase and for transferrin, although the esterase was nearly monomorphic.

We interpret the *GPI-A** differentiation as being indicative of stock differences, resulting from restricted gene exchange between the four identified regions. However, the alternative explanation, that of differential selection in the presence of gene flow, cannot be ruled out. Indeed, the very limited mtDNA differentiation observed could be held to support this interpretation. Microsatellite analysis, currently underway, may help to resolve this question. Selection acting on these noncoding genetic markers is presumed to be minimal or nonexistent; therefore microsatellite differentiation paralleling the *GPI-A** differentiation would suggest drift of neutral *GPI-A** alleles, whereas lack of microsatellite differentiation would indicate significant gene flow and thereby implicate selection as the cause of the *GPI-A** differentiation. Pogson et al. (1995) have recently suggested that the highly heterogeneous distribution of anonymous nuclear RFLP markers among populations of cod, *Gadus morhua*, reflects limited gene flow and that the much more homogeneous distribution of allozyme alleles reflects stabilizing selection rather than extensive gene flow. Such an argument applied to yellowfin tuna data would interpret the *GPI-A** heterogeneity as indicative of limited gene flow, and the *ADA**, *FH**, and *GPI-B** homogeneity as indicative of stabilizing selection at these three loci.

Differences between collections in mtDNA was only just significant (P=0.048), with a "true" G_{ST} value across all nine collections of around 1%. When collections were pooled within oceans, i.e. the three groups (Atlantic, Indian, and Pacific), significant differentiation was detected (P=0.009), although the "true" G_{ST} was only of the order of 0.5%. All three pairwise ocean comparisons were statistically significant. However, because collections within oceans did not always pool together in the distance dendrograms (Fig. 3), possibly because of limited sample sizes, it would clearly be useful to have more data to confirm (or refute) this evidence of interoceanic differentiation. When collections were pooled into the four putative stocks indicated by the *GPI-A** data, limited but significant heterogeneity in mtDNA haplotype frequencies was apparent (P=0.024), but there were no significant pairwise comparisons.

Scoles and Graves (1993) were unable to detect significant mtDNA differentiation between Pacific and Atlantic yellowfin tuna, whereas the probability of homogeneity in our tests of these two oceans was only 0.017. However, they adopted a different test strategy. Instead of examining relatively large numbers of fish (our study: Pacific fish, n=561; Atlantic fish, n=94) with relatively few restriction enzymes (n=2, but known to detect polymorphic sites), they chose to examine relatively few fish (Pacific fish, n=100; Atlantic fish, n=20) with a relatively large number of restriction enzymes (n=12, which included the two enzymes we used). Given that the common 12-enzyme haplotype in Scoles and Graves' study comprised 52 fragments or 304 bp and that the common 2-enzyme haplotype in our study comprised 7 fragments or 42 bp (see Ward et al., 1994) and that the mean size of the yellowfin tuna mtDNA genome is about 16,702 bp (Scoles and Graves [1993] estimate=16,549; Ward et al. [1994]=16,856), Scoles and Graves surveyed about 1.8% of the mtDNA genome, whereas we surveyed only about 0.3%. However, although it is of course true that had we surveyed more restriction enzymes, we would have uncovered many additional haplotypes, the two enzymes that we did select revealed most of the mtDNA diversity shown by Scoles and Graves (1993). For example, the (pooled) 12-enzyme haplotype diversity of 0.840 of Scoles and Graves was not much larger than our (pooled) 2-enzyme diversity of 0.677. Four of the enzymes used by Scoles and Graves showed no variation at all in the 120 fish and therefore were of no use for population discrimination. Twenty of the 34 12-enzyme haplotypes detected by Scoles and Graves (1993) among their 120 fish were seen only once, whereas only four of the 22 2-enzyme haplotypes in our 655 Atlantic and Pacific fish were seen only once: such rare haplotypes are of extremely limited use in population studies. Given that mtDNA heterogeneity among regions is very limited, it is not surprising that the approach of screening large numbers of fish for a small number of sequences known to be variable should be more powerful than screening small numbers of fish for a larger number of sequences, many of which are relatively invariant.

MtDNA data from another tuna, the albacore, *Thunnus alalunga*, showed a somewhat more pronounced separation of Atlantic Ocean and Pacific Ocean collections than did data for yellowfin tuna, but again no intraoceanic heterogeneity was detected (Chow and Ushiama, 1995).

The limited mtDNA differentiation among yellowfin tuna sampled throughout their range contrasts with the marked population subdivision revealed by the *GPI-A** locus. Mitochondrial DNA has an effective population size only one quarter that of nuclear DNA (Birky et al., 1989) and evolves more rapidly

(Brown et al., 1979); in principle it should be a more effective indicator of population substructure than nuclear loci. Given that more mtDNA than nuclear DNA divergence is expected, how can an allozyme locus show differentiation when mtDNA haplotypes do not? The lack of mtDNA differentiation in yellowfin tuna does not appear to be the result of a lack of variation nor of a small sample size: although increasing haplotype diversities and sample sizes will increase statistical power, the mtDNA haplotype diversities of our populations, assayed for just two restriction enzymes, were quite high at around 0.65–0.70, and sample sizes were similar to those used in the allozyme analyses. Nuclear DNA differentiation can exceed mtDNA differentiation when either the migration rate or the breeding sex ratio is strongly biased towards females (because mtDNA is maternally inherited), but there is no evidence that either of these conditions holds for yellowfin tuna (e.g. IATTC, 1992). The explanation for the seeming discrepancy may be that several independent polymorphic allozyme loci were screened, whereas haplotypes of mtDNA are best treated as alleles at a single, nonrecombining locus. In a situation of low overall genetic divergence (resulting from gene flow or recent separation), the stochastic nature of genetic drift means that if several allozyme loci are screened, and notwithstanding the expected higher rate of mtDNA evolution, divergence might be first detected at an allozyme locus before it is detected for mtDNA. An alternative explanation, as intimated earlier, is that the *GPI-A** differentiation results from selection.

The delineation of the four stocks of yellowfin tuna does not seem unreasonable given what we know of their distribution and movements. Yellowfin are found circumglobally, but only in tropical and subtropical oceanic waters, approximately between the latitudes 40°N and 40°S (Collette and Nauen, 1983). Spawning occurs throughout the year in all core areas of distribution, peaking in the warmer months (Collette and Nauen, 1983). Waters off the southern regions of South America (approximately 55°S) are too cold for Atlantic Ocean and Pacific Ocean fish to migrate around Cape Horn. Furthermore, direct connections between the tropical Atlantic Ocean and the eastern Pacific Ocean were severed after the Isthmus of Panama closed about 3.5 million years ago (e.g. Keigwin, 1982; Coates et al., 1992), a closure likely to have predated the origin of yellowfin tuna (estimated by Elliott and Ward (1995) to have occurred within the last two million years). Thus Pacific Ocean and Atlantic Ocean fish could not mix. In contrast, Atlantic Ocean and Indian Ocean fish could mix (through southern Africa waters), as could Indian Ocean and Pacific Ocean fish (through Indonesian waters), but tagging experiments indicate that most yellowfin tuna move on a scale of hundreds rather than thousands of kilometers (Joseph et al., 1964; Bayliff, 1979; Hunter et al., 1986; Lewis, 1992). The extent of migration between ocean basins is therefore likely to be low, with intraoceanic recruitment predominating. Nonetheless, interoceanic movements are possible and could account for the low degree of genetic differentiation among areas. Further discussion of the genetic and other biological data with respect to Pacific Ocean fish is given in Ward et al. (1994).

At present, these suggestions on the global stock structure of yellowfin tuna are essentially based on gene frequencies at a single polymorphic allozyme locus, *GPI-A**, because no significant genetic heterogeneity was detected for three other polymorphic allozymes and the mitochondrial DNA variants showed little interpopulation differentiation. It may well be that the stock structure of yellowfin tuna, in management terms, is more complex than these present findings suggest: very limited migration between areas can effectively homogenise gene frequencies, and thus dispersal between areas can still be low even between populations that cannot be genetically discriminated.

Future genetic work should include the examination of more fish from the Indian Ocean because the identification of these fish as a separate stock is based primarily on the analysis of just 21 fish for a single allozyme locus. Further clarification of genetic stock structure issues in yellowfin tuna will require larger sample sizes, examination of more areas (especially from the Indian and Atlantic Oceans), and the deployment of genetic techniques, such as microsatellite analysis, with enhanced resolving power and less concern over neutrality and selection issues.

Acknowledgments

This work was supported by grant 91/27 from the Fisheries Research and Development Corporation. We thank the following who assisted us by collecting samples: Noel Barut, Barbara Block, Chris Boggs, Thor Carter, Tim Davis, Ed Everett, John Gunn, Aubrey Harris, Nishi Karunasinghe, Theresa O'Leary, Dennis Lee, Dave Milton, Pianet Renaud, and Toki Takenaka. Shirlena Soh helped with some of the allozyme analyses. Chris Bolch, Rene Vaillancourt, Vivienne Mawson, Peter Rothlisberg, and two anonymous referees made useful comments on a draft of this manuscript.

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