Allozyme and RAPD variation in the eastern Pacific yellowfin tuna (*Thunnus albacares*)

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Abstract—Stock structure of eastern Pacific yellowfin tuna was investigated by analyzing allozymes and random amplified polymorphic DNAs (RAPDs) from 10 samples of 20–30 individuals each, collected between 1994 and 1996 from fishing vessels operating in the Inter-American Tropical Tuna Commission (IATTC) yellowfin regulatory area (CYRA). Allozyme analysis resolved 28 loci, eight of which were polymorphic under the 0.95 criterion: *Aat-S**, *Glud*, *Gpi-F**, *Gpi-S**, *La*, *Lgg*, *Pap-F**, and *6-Pgd*, resulting in a mean heterozygosity over all allozyme loci of $H = 0.052$. Four polymorphic RAPD loci were selected for analysis, resulting in a mean heterozygosity of $H = 0.43$. Eight of 45 pairwise comparisons of allozyme allele frequencies among the ten samples showed significant differences after correction for multiple testing ($P<0.0001$), all of which involved comparisons with the Gulf of California sample. Confirmation of this signal of population structure would have management implications. No significant divergence in RAPD allele frequencies was observed among samples. Weir and Cockerham θ estimated for allozyme loci ($\theta=0.048$; $P<0.05$) and RAPD loci ($\theta=0.030$; $P>0.05$) revealed little population structure among samples. Mantel tests demonstrated that the genetic relationships among samples did not correspond to an isolation-by-distance model for either class of marker. Four of eight comparisons of coastal and offshore samples revealed differences of allele frequencies at the *Gpi-F** locus ($P<0.05$), although none of these differences was significant after correction for multiple testing ($P>0.001$). Results are consistent with the hypothesis that the CYRA yellowfin tuna samples comprise a single genetic stock, although gene flow appears to be greater among coastal samples than between coastal and offshore samples.

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Yellowfin tuna (*Thunnus albacares*) is a cosmopolitan species inhabiting tropical and subtropical waters in the Atlantic, Pacific, and Indian oceans. This species has accounted for more than a third of the world's tuna production since 1970. The eastern Pacific has contributed from 21% to 26% of the global catch from 1993 through 1997, representing 273,329 metric tons (t) in 1990 to 264,426 t in 1998 (IATTC, 1999).

Yellowfin tuna is a large pelagic fish with a common size of 150 cm (Collette and Nauen, 1983). Spawning occurs throughout the year in the tropical oceans, preferably near islands and coasts (Leis et al., 1991). Growth is rapid and individuals reach maturity by the end of the second year (Suzuki et al., 1978). Schooling of individuals of similar size is observed near surface waters and is often associated with floating objects (Wild, 1994).

Yellowfin tuna is currently considered to comprise a single species (Gibbs and Collete, 1967), although significant morphometric and meristic differences, limited fish movements, and differences among catch data, have been reported for the different regions of the Pacific Ocean (Godsil and Greenhood, 1951; Schaefer, 1955; Joseph et al., 1964; Suzuki et al., 1978; Schaefer, 1991). Population structure in yellowfin tuna has been addressed in the Pacific Ocean by using several independent methods. Morphometric and meristic based studies have shown significant differences (Godsil and Greenhood, 1951; Schaefer, 1955; Kurogane and Hiyama, 1957), and at least three stocks or discrete units (western, central, and eastern Pacific) have been proposed. More recent studies using morphometric multivariate analysis suggest the presence of different stocks between north and south regions in the eastern Pacific (Schaefer, 1991), as well as across the Pacific Ocean (Schaefer, 1991). Additionally, differences in larval distribution, catch rates, and size composition data of yellowfin tuna caught along the equatorial Pacific by longline and purse-seine have been used by Suzuki et al. (1978) to distinguish between western, central, and eastern Pacific groups.

Tagging experiments have shown limited movement of yellowfin tuna between western and eastern Pacific waters (Joseph et al., 1964; Fink and Bayliff, 1970). In the eastern Pacific, the presence of two groups has been suggested: a northern group off Baja California coast and the Revillagigedo Islands and a southern group from the Maria Islands through Chile. Some mixing occurs between them (Fink and Bayliff, 1970). There seem to be marked movements between north and south groups along the coast with limited westward movements (Joseph et al., 1964).

Some studies of population structure using genetic analyses have not revealed the presence of discrete stocks along the Pacific Ocean. Barret and Tsuyuki (1967) used transferrin analysis and did not find differences in allele frequencies between samples from Hawaii and eastern Pacific samples, although heterogeneity was detected within the eastern Pacific samples (IATTC, 1975). Allozyme variation studies in the esterase locus (Fujino, 1970) did not show enough evidence of genetic differentiation between eastern Pacific and Hawaii samples. Furthermore, Scoles and Graves (1993) used restriction fragments length polymorphisms (RFLP) and analysis of mitochondrial (mt) DNA to examine five samples collected across the Pacific Ocean and one from the Atlantic Ocean. Although they detected 34 haplotypes and considerable genetic variation, no evidence of genetic differentiation among samples was found.

However, more recent genetic studies have provided limited evidence of genetic heterogeneity. Ward et al. (1994) analyzed four polymorphic allozyme loci and 18 mtDNA haplotypes in yellowfin tuna from the Pacific Ocean. Although no unique haplotypes were found in the analyzed populations through RFLP analysis, the eastern Pacific samples were found to be different from the central and western Pacific samples in frequency differences at a single locus *GPI-F*,* suggesting that the signal of population structure exhibited is due to selective factors contributing to the divergence. Eastern Pacific samples (n=41) were collected in the northeast Pacific off California and at an unspecified site off Mexico (n=40). Comparisons of *GPI-F** allele frequencies from eastern Pacific also included two samples previously analyzed by Sharp (1978) from Roca Partida (Central America) and Ecuador. Their results showed population homogeneity at the *GPI-F** locus for this region.

To date, the methods and logistics used to study divergence in the Pacific yellowfin tuna have been focused on a global rather than a local scale, and sampling has been focused on the wide areas of the west and central Pacific. Local structure in the eastern Pacific yellowfin tuna has not been addressed through a more intense sampling strategy to examine genetic homogeneity in this region. Because tagging studies have shown restricted longitudinal movements by yellowfin tuna, population structure and isolation by distance hypotheses can be tested. To evaluate the stock structure of yellowfin tuna in eastern Pacific, we employed analyses of allozymes and of randomly amplified polymorphic DNA (RAPDs).

RAPDs have proven to be useful genetic markers because of their high levels of polymorphisms (Williams et al., 1990; Welsh et. al., 1991). They have been used to estimate population structure in fishes, including the cod (Kenji, 1998), red mullet (Mamuris et al., 1998), and striped bass (Bielawski and Pumo, 1997). The use of RAPDs, considered as neutral markers, and the simultaneous use of allozyme analyses with intense sampling in a more local area, might provide evidence about the relationship between gene flow and spatial distribution of the eastern Pacific yellowfin tuna, as well as evidence of the presence of local selective factors responsible for the divergence suggested by Ward et al. (1994).

Materials and methods

Sampling

A total of 327 tissue samples from specimens of ten locations were obtained from commercial tuna boats fishing in the tropical eastern Pacific from 1994 to 1996 (Fig. 1). Muscle tissue samples were dissected from specimens at the time of landing and were transported in liquid nitrogen or on dry ice to the Laboratorio de Genética de Organismos Acuáticos of the Instituto de Ciencias del Mar y Limnología in Mexico City. Samples were maintained at –70ºC until processing.

Allozyme analysis

For allozyme analysis, 1 cm^3 (about one gram) of tissue sample was ground with a manual homogenizer in 1.5 mL of extraction buffer (0.01M Tris-0.001M EDTA, pH 6.8, and 1% NADP) and centrifuged at 2500 g at 4°C. Electrophoretic runs were performed in 12% (w/v) starch gels (Sigma Chemicals, St. Louis, MO). Four buffer systems were used to analyze nineteen enzymes that resolved 28 loci, eight of which showed polymorphism: *Aat-S** (aspartate aminotransferase), *Glud* (glutamate dehydrogenase), *Gpi-F** and *Gpi-S** (glucose phosphate isomerase), *La* (leucil-L-alanine), *Lgg* (L-leucil-glycil-glycine), *Pap-F** (L-leucil-L-proline) and *6-Pgd* (phosphogluconate dehydrogenase). Enzymes AK (adenilate kinase), CK (creatinine kinase), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), LDH (lactate dehydrogenase), MDH (mMalate dehydrogenase), ME (malic enzyme) and SOD (superoxide dismutase), displayed twenty more loci that were presumably monomorphic. Buffer systems for enzyme analysis were 1) amino-citrate: 0.04M citric acid, 15mL/L of N-3-aminopropyl-morpholine, pH 6.5 (AAT, GPI, and LA); 2) 0.008M Tris, 0.003 M citric acid, pH 6.7 (GLUD and 6-PGD); 3) 0.025 M Tris, 0.192 glycine, pH 8.5 (GPI and LGG); 4) 0.0.076 M Tris, 0.005 M citric acid, pH 8.7 (PAP). Enzyme assays were performed following Harris and Hopkinson (1976). Enzymes showing polymorphism were analyzed for all samples and subjected to population genetic analysis.

RAPD analysis

For RAPD analyses, genomic DNA was extracted from muscle tissue by using standard phenol-chloroform protocols (Sambrook et al., 1989), resuspended in TE buffer (10mM Tris-0.1mM EDTA pH 8.0), and quantified with a Hoefer DyNA quant 200 fluorometer. DNA was amplified with primer F-10 (Operon® Alameda, CA; 5′-GGAAGCTTGG-3′). Amplifying reactions were performed in a final volume of 22 μL consisting of 0.7 to 1 ng/μL of DNA in amplification buffer, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 33 ng of primer, 10 mM dNTPs, and 1 U of Taq polymerase. Amplification of genomic DNA was performed in a Perkin Elmer®, Foster City, CA (mod. 480), thermal cycler. The program was set for 1 cycle of 1 min. at 36°C, followed by 44 cycles of 1 min. at 36°C; 1 min. at 94°C; 2 min. at 72°C, and

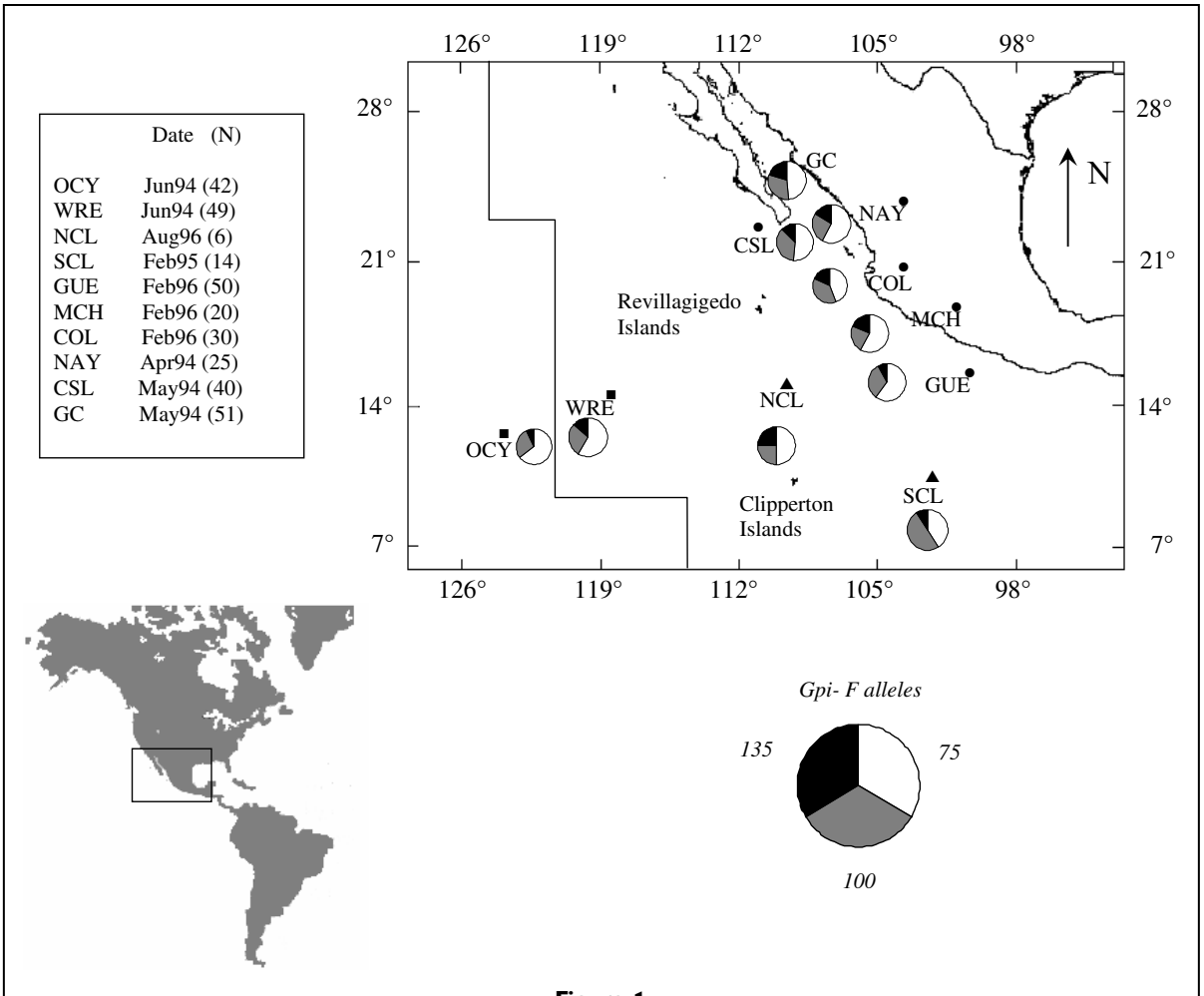

Figure 1

Thunnus albacares. Location of sampling sites, dates, and sample sizes, and *Gpi-F** gene frequencies of eastern Pacific yellowfin tuna. The sites are as follows: OCY = out of CYRA area; WRE = west of the Revillagigedo Islands; NCL = North Clipperton islands; SCL = South Clipperton Islands; MCH = Michoacan; GUE = Guerrero; COL = Colima; CSL = Cape San Lucas; NAY = Nayarit; GC = Gulf of California. Symbols are ● coastal, ▲ intermediate, ■ offshore.

a final cycle of 15 min. at 72°C. Optimal DNA concentrations for amplification were determined by testing several dilutions, one of which was taken as the standard for every subsequent amplification.

Amplified fragments were resolved by electrophoresis in 1.5% agarose gels (Sigma Chemicals) for 3 to 4 h. at 90 mA (100 V). A 100bp DNA Ladder (GibcoBRL, Gaithersburg, MD, 15628-019) was used as size standard. After electrophoresis, gels were stained with ethidium bromide and photographed in a UV light transilluminator.

Data analysis

Allelic frequencies, test of conformity of genotype distributions with Hardy-Weinberg, and heterozygous deficit were determined by using Genepop version 3.3 (Raymond and Rousset[1]). Homogeneity of allozyme and RAPD allele frequencies was evaluated by using the exact probability test (Raymond and Rousset, 1995) consisting of a contingency analysis for every polymorphic locus and an estimation of their probability values by the combined probability of Fisher (Sokal and Rohlf, 1995) as implemented in the TFPGA program (Miller[2]). Pairwise comparisons were conducted to determine allele frequency differences among samples in order to define sources of variation. Based on the longitudinal differentiation pattern observed by Ward et al. (1994) and the morphological latitudinal differences within eastern Pacific samples reported by Schaefer (1991)

[1] Raymond, M. L., and F. Rousset. 1995a. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicist. J. Heredity 86:248–249.

[2] Miller, M. P. 1997. Tools for genetic populations analyses (TFPGA) 1.3: a windows program for the analysis of allozyme and molecular population genetic data, 29 p. Computer software distributed by the author at http://bioweb.usu.edu/mpmbio.

at north-south of the 15–20°N range, spatial homogeneity was tested at the following levels: overall samples (O), among longitudinal regions (L; coastal, intermediate, and offshore localities), and latitudinal regions (N; north-south of the 15–20° range). The Gulf of California sample was excluded from this analysis because the large variation found in this sample (reflected in the significant differences shown in allele frequency homogeneity from pairwise comparisons) would not allow an accurate assessment of whether longitudinal or latitudinal differentiation exists or not. Significance levels were adjusted for multiple testing through the Bonferroni sequential method (Rice, 1989).

Population subdivision was estimated by using the Weir and Cockerham (1984) method through the TFPGA program. Standard error and confidence intervals were obtained through jackknife and bootstrapping procedures, respectively, with F_{ST} Pro 1.0 (Weir, 1990). Estimates of population subdivision were partitioned into the following levels: O, over all the samples; L, longitudinal regions (coastal, intermediate and offshore); and N, latitudinal regions.

We used the θ statistic to estimate gene values between sample pairs (Slatkin, 1993) that are defined as M_θ. An "isolation by distance" model was evaluated from the correlation between the distance between localities measured as geographic separation in nautical miles (nmi), and the M_θ values by means of the Mantel test (Hellberg, 1994) in both allozymes and RAPDs.

The patterns of the amplification products resulting from the RAPD analysis were subjected to the same analyses as allozymes with the procedures described in Lynch and Milligan (1994). RAPD fragments were interpreted under the following assumptions: 1) fragments were considered to behave as dominant genes (Williams et al., 1990); 2) every polymorphic fragment was considered derived from a two-allele locus; 3) the equilibrium of Hardy-Weinberg was assumed for all genotypes, and 4) each fragment was considered to be an independent locus.

Only those fragments clearly defined and having consistent intensity were recorded. Because of this, the Michoacan sample, with poor consistency in the banding patterns, was excluded from the RAPD analysis. The allele frequency of every fragment was calculated on the basis of the inferred homozygous recessive genotypes. Because of the dominant nature of the alleles, and in order to correct the bias originated by calculating the recessive allelic frequencies, we chose the estimation based on the Taylor expansion (Kendall and Stuart, 1977, cited in Le Corre et al., 1997) as implemented in TFPGA program. This reduction on the bias is based on the equation resulting from the second order expansion of Taylor (see details in Lynch and Milligan, 1994).

Results

Allozymes

Of the 28 analyzed allozyme loci, eight (28.5%) showed polymorphism under the 0.95 criterion. The observed heterozygosities per sample over all allozyme loci ranged from 0.027 to 0.083 (mean 0.052). Allozyme frequencies for the eight polymorphic loci detected are shown in Table 1. After adjusting levels of significance by the Bonferroni procedure, significant deviation of genotypic frequencies from those expected under Hardy-Weinberg was found in the loci *Lgg* and *Pap-F** for the Gulf of California sample and in the *Aat-S** locus for two localities—west of Revillagigedo Islands, and the Gulf of California (P<0.0006, Table 1). Deviations displayed for both locations corresponded to a heterozygous deficit (P<0.0006, after Bonferroni correction).

Comparison of allozyme allele frequencies among all collections (overall) by the exact probability test revealed significant heterogeneity at loci *Glud*, *La,* and *Lgg*, after Bonferroni adjustment (P<0.006). Pairwise comparisons among samples to test allele homogeneity gave significant differences for nine of 45 comparisons after correction for multiple tests (P<0.001), seven of which involved comparisons with the Gulf of California (GC) sample. The remaining significant differences were between Guerrero-Nayarit, and Cape San Lucas-Nayarit comparisons (Table 2), resulting from significant heterogeneity at *Glud*, *La,* and *Lgg* loci.

In general, allozyme analysis displayed low levels of differentiation. The θ value over all loci was different from zero (P<0.05) and showed that 4.8% of the variance was attributable to differences among samples (Table 3).

Individual loci showed θ values ranging between 0.0037 and 0.27. Highly significant values at loci *La* (θ=0.13 ±0.089) and *Lgg* (θ=0.27 ±0.253) evidently resulted from their weak polymorphism in some samples (Table 3).

Allele frequency homogeneity was tested among coastal, intermediate, and offshore regions (Table 4). Significant heterogeneity was found by exact test between coastal and offshore comparisons (P=0.0043) but was found to be not significant between coastal and intermediate regions (P=0.0632). Subdivision as measured by θ among coastal, intermediate, and offshore localities was not different from zero. However, for the *Gpi-F** locus the population subdivision among regions (0.0058) was twice as large as that noted among samples (θ=0.003), but neither value was significant (Table 3). No latitudinal differentiation by the exact test or population subdivision estimated by the θ index was found between north and south regions (data not shown).

The gene flow values (M_θ) were high (mean 24.8 migrants per generation). A lack of correlation between gene flow estimations and geographic distance by means of the Mantel test was observed (r^2=–0.144; P=0.22), resulting in a rejection of the isolation by distance model.

For the *Gpi-F** locus, paired tests of significance (data not shown) showed discrepancies in the *Gpi-F*/75* allele frequencies among localities from the coast with those located at the CYRA limits (Fig. 1, Table 5). Four of eight comparisons of coastal and offshore samples revealed differences of allele frequencies at this locus (P<0.05), although none of these differences was significant after correction for multiple testing (P>0.001).

RAPDs

The primer OPF-10 produced 11 amplified fragments, with sizes from 200 to 600 bp (base pairs). Four of the fragments

Table 1

Allele frequencies for allozymes and RAPDs, samples size (n) and agreement to the Hardy-Weinberg equilibrium (HW) for every loci and sample of *Thunnus albacares*. Significance values for HW tests were adjusted for multiple comparisons with an initial α level of 0.0006 [(0.05/(8 loci × 10 samples)]. P = probability of significance for allele frequency heterogeneity per locus, * = significant at α = 0.006. — = no data. OCY = out of CYRA area; WRE = west of the Revillagigedo Islands; NCL = North Clipperton islands; SCL = South Clipperton Islands; MCH = Michoacan; GUE = Guerrero; COL = Colima; CSL = Cape San Lucas; NAY = Nayarit; GC = Gulf of California.

		Collection										
Locus	Allele	OCY	WRE	NCL	SCL	GUE	MCH	COL	CSL	NAY	GC	P
*Aat-S**	–90	0.171	0.266	0.250	0.231	0.154	0.275	0.077	0.092	0.0	0.234	
	–100	0.829	0.734	0.750	0.769	0.846	0.725	0.923	0.908	1.0	0.766	
	n	41	32	6	13	39	20	26	38	25	47	0.011
	HW	yes	no	yes	yes	yes	yes	yes	yes	—	no	
Glud	100	0.700	0.634	0.667	0.857	0.663	0.775	0.914	0.788	0.680	0.395	
	85	0.300	0.366	0.333	0.143	0.337	0.225	0.086	0.212	0.320	0.605	*
	n	40	41	6	14	49	20	29	40	25	43	
	HW	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	
*Gpi-F**	135	0.071	0.134	0.250	0.091	0.190	0.079	0.185	0.125	0.160	0.200	
	100	0.262	0.280	0.250	0.500	0.230	0.316	0.370	0.363	0.260	0.314	
	75	0.667	0.586	0.500	0.409	0.580	0.605	0.444	0.512	0.580	0.486	0.526
	n	42	41	6	11	50	19	27	40	25	35	
	HW	yes	yes	yes	yes	yes	no	yes	yes	yes	yes	
*Gpi-S**	–60	0.402	0.536	0.417	0.571	0.360	0.425	0.414	0.449	0.479	0.338	
	–100	0.598	0.464	0.583	0.429	0.640	0.575	0.586	0.551	0.521	0.663	0.015
	n	41	42	6	14	50	20	29	39	24	40	
	HW	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	
La	120	0.0	0.024	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	
	100	1.0	0.976	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.8	*
	n	42	42	6	14	50	20	24	40	20	50	
	HW	yes	—	—	—	—	—	—	—	yes	yes	
Lgg	115	0.0	0.024	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.349	
	100	1.0	0.976	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.651	*
	n	42	42	6	14	50	20	24	24	24	43	
	HW	yes	—	—	—	—	—	—	—	—	no	
*Pap-F**	110	0.0	0.0	0.0	0.0	0.02	0.05	0.0	0.0	0.0	0.083	
	100	1.0	1.0	1.0	1.0	0.98	0.95	1.0	1.0	1.0	0.917	0.055
	n	42	42	6	14	50	20	29	40	25	48	
	HW	—	—	—	—	yes	yes	—	—	—	no	
6-Pgd	100	0.902	0.903	0.667	0.821	0.939	1.0	0.966	1.0	0.840	0.938	
	90	0.098	0.097	0.333	0.179	0.061	0.0	0.034	0.0	0.160	0.062	0.007
	n	41	31	6	14	49	20	29	40	25	48	
	HW	yes	yes	yes	yes	yes	—	yes	—	yes	yes	
F10-1	*a*	0.406	0.282	0.234	0.322	—	0.083	0.505	0.355	0.282	0.246	
	b	0.594	0.718	0.766	0.678	—	0.917	0.495	0.645	0.718	0.754	0.572
	n	9	8	7	9	—	6	9	10	8	9	
	HW	—	—	—	—	—	—	—	—	—	—	
F10-2	*a*	0.406	0.603	0.441	0.322	—	0.278	0.322	0.355	1.0	0.322	
	b	0.594	0.397	0.559	0.678	—	0.722	0.678	0.645	0.0	0.678	0.369
	n	9	8	7	9	—	6	9	10	8	9	
	HW	—	—	—	—	—	—	—	—	—	—	
F10-3	*a*	0.406	0.475	0.329	0.625	—	0.397	0.406	0.529	0.371	0.406	
	b	0.594	0.525	0.671	0.375	—	0.603	0.594	0.471	0.629	0.594	0.912
	n	9	8	7	9	—	6	9	10	8	9	
	HW	—	—	—	—	—	—	—	—	—	—	
F10-4	*a*	0.115	0.282	0.234	0.178	—	0.083	0.246	0.219	0.138	0.246	
	b	0.885	0.718	0.766	0.822	—	0.917	0.754	0.781	0.862	0.754	0.929
	n	9	8	7	9	—	6	9	10	8	9	
	HW	—	—	—	—	—	—	—	—	—	—	

Table 2

Pairwise-sample comparisons of allele frequency homogeneity for *Thunnus albacares*. Probability values in allozymes are above the diagonal (—) and RAPDs are below the diagonal (—). * = significant values after Bonferroni correction for multiple tests (initial α was 0.05). na = data not available. OCY = out of CYRA area; WRE = west of the Revillagigedo Islands; NCL = North Clipperton islands; SCL = South Clipperton Islands; MCH = Michoacan; GUE = Guerrero; COL = Colima; CSL = Cape San Lucas; NAY = Nayarit; GC = Gulf of California.

Sample	OCY	WRE	NCL	SCL	GUE	MCH	COL	CSL	NAY	GC
OCY	—	0.5807	0.7391	0.3414	0.4386	0.8254	0.0295	0.1525	0.0194	*<0.001
WRE	0.9316	—	0.9482	0.6047	0.4330	0.1180	0.0012	0.0084	0.0192	*<0.001
NCL	0.9797	0.9770	—	0.9232	0.3494	0.7307	0.1567	0.0859	0.4779	0.0174
SCL	0.9798	0.8088	0.8915	—	0.3989	0.0717	0.3414	0.1093	0.0287	*<0.001
GUE	0.5935	0.4732	0.9095	0.7757	—	0.4137	0.1723	0.5932	*<0.001	*<0.001
MCH	na	na	na	na	na	—	0.0500	0.1532	0.0096	*<0.001
COL	0.9385	0.7864	0.9155	0.9794	0.4569	na	—	0.8237	0.0082	*<0.001
CSL	0.9475	0.9703	0.9824	1.000	0.6576	na	0.9849	—	0.0012	*<0.001
NAY	0.9656	0.9984	0.8418	0.4965	0.4101	na	0.4206	0.5707	—	*<0.001
GC	0.9458	0.9637	0.9987	0.9977	0.9743	na	0.9715	0.9874	0.6518	—

Table 3

Estimates of population subdivision θ (Weir and Cockerham, 1984) for allozymes of *Thunnus albacares* partitioned into longitudinal regions θ_L (regions) (i.e. coastal-intermediate-offshore) and samples θ_0 (overall). n.v. = negative values. *P* = probability of significance of subdivision estimations. Significance of single-locus values was corrected with an initial level of 0.006 (0.05/8 loci). Means and standard error were obtained by the jackknife method. Confidence intervals obtained by 1000 resamplings through bootstrapping are also shown. * = signifcant values after Bonferroni correction.

Locus	$\theta_{L\,(\text{regions})}$	*P*	$\theta_{0(\text{overall})}$	*P*
*Aat-2**	0.0152	0.072	0.029	0.007
Glud	0.0009	0.227	0.086	>0.006*
*Gpi-1**	0.0058	0.086	0.003	0.210
*Gpi-2**	0.0014	0.780	0.0037	0.007
La	n.v.	0.012	0.13	>0.006*
Lgg	0.0073	0.004	0.27	>0.006
*Pap-1**	n.v.	0.068	0.024	0.001
6-Pgd	0.044	>0.006*	0.02	>0.006*
Mean	0.0067 ±0.0042		0.048 ±0.022	
CI 95%	0.0003–0.0199		0.019–0.101	

Table 4

Pairwise-regions comparison of allele frequencies for *Thunnus albacares*. Probabilities of nonheterogeneity for allozymes (based on exact tests) are above the diagonal (—) and RAPDs are below the diagonal (—). * = significant after corrected for multiple tests (Rice, 1989).

Region	Coastal	Intermediate	Offshore
Coastal	—	0.0632	0.0043*
Intermediate	0.9998	—	0.5384
Offshore	0.9039	0.9126	—

were polymorphic for all samples (Table 1). No significant heterogeneity of RAPD allele frequencies was found for any locus between any paired sample comparison, among all collections, nor among latitudinal or longitudinal regions (*P*=0.4806).

The mean θ value for all fragments and samples (overall), as well as regional estimations derived from RAPDs (0.0302), were not significantly different from zero and displayed some negative values. Estimations of gene flow between sample pairs (M_θ) from RAPD data averaged 29.2 migrants per generation. The evaluation of the relationships between geographic distances and the gene flow estimations in pairwise collections (M_θ), through the Mantel test, showed a nonsignificant correlation (r^2=0.413, *P*=0.984).

Discussion

The test of conformance to the Hardy-Weinberg frequencies showed significant differences in *Lgg* and *Pap-F** loci only in the Gulf of California sample, where polymorphism at those loci was also consistently found. Similar results were obtained, with smaller differences in locus *Aat-S** from the west of the Revillagigedo Islands and the Gulf of California samples. Considering the fact that our samples were provided by the commercial fleet, they could have included representatives of different schools with differences in genotypic distributions originated by differences in age classes or sexual ratios (or for both) among schools because recruitment of individuals into new schools has been reported to be mainly by aggregating individuals of

Table 5

Comparison of *Gpi-F** allele frequencies for *Thunnus albacares* among data from the present study and those reported in Ward et al. (1994). — = data absent.

Locus Allele	Western/Central[1]	Eastern[1]	Eastern (present data)			
			Offshore	Intermediate	Coastal	Pooled
*Gpi-F**						
135	0.026	0.100	0.103	0.147	0.163	0.145
100	0.640	0.269	0.301	0.412	0.301	0.307
75	0.332	0.631	0.596	0.441	0.566	0.548
40	0.002	—	—	—	—	—
n	346	178	83	17	196	296

[1] Allele frequencies for *Gpi-F** reported in Ward et al. (1994).

similar sizes (Collette and Nauen, 1983). Because recruitment to the original tuna schools has been reported as well (Kimley and Holloway, 1999), random processes could also induce differences in genotypic frequencies that favor aggregation of some genotypes, while segregating some others, causing a kind of Wahlund effect that is reflected by a heterozygous deficit as shown by the homozygous excess for loci and locations having HW deviations, especially as shown in the Gulf of California sample.

The estimations of population structure based on allozymes showed a small but significant value different from zero (θ=0.048; P<0.01). The Gulf of California sample contributed to the significant subdivision value as shown when that collection was excluded from the regional subdivision analysis, as well as to significant heterogeneity of its allele frequencies when paired comparisons were made.

The small value of θ for overall estimations on RAPD data is probably due to the small sample size. The negative values of θ from overall and regional estimations resulted from subtracting the large value of the correction derived from the variation expected of the sample sizes from the small value of variation due to fluctuations in allele frequencies. The fact that RAPD data are considered dominant could reduce information about the true allele distributions by subestimation of null allele frequencies notwithstanding the correction applied to recessive genotypes, which is dependent on the sample sizes (Lynch and Milligan, 1994). Other assumptions for RAPD data limit the value of this marker, especially when estimations are derived from a small number of loci and sample sizes. Additional constraints are related to the limited number of alleles (two) to estimate dominant markers, which tend to subestimate the polymorphism and thus reduce the significance of relatively small discrepancies in allele distributions.

No differentiation between coastal and offshore samples was found in our study because of the slight, nonsignificant differences in the estimation of the subdivision by regions. Although the overall estimation was not different from zero, the allele homogeneity analysis showed allele-frequency heterogeneity between coastal and offshore samples, and nonheterogeneity between coastal and intermediate samples.

These results are consistent with the migration reports through tagging studies; evidence exists for the presence of two main yellowfin tuna groups in the eastern Pacific that mix to some extent (Fink and Bayliff, 1970) and that migrate longshore from around the 20°N to the mouth of the Gulf of California and to the zone between the Revillagigedo and the Clipperton islands, and back again (Joseph et al., 1964; Fink and Bayliff, 1970), although longitudinal movements are restricted to the limits of yellowfin regulatory area (CYRA). Similarly, important northward movements along the coasts to the mouth of the Gulf of California, and subsequently to the western coasts of Baja California, have been reported. Although the estimation of θ for allozymes showed a significant value, it was notably influenced by the heterogeneity found between the Gulf of California sample and all other samples.

Discarding the variation displayed by loci *La*, *Lgg,* and *Pap-F**, originating mainly from Gulf of California sample, the estimation of subdivision was still marginally significant after Bonferroni correction, which should be considered as evidence that the Gulf of California sample may represent a partially isolated population with different allele frequencies. Oceanographic conditions inside the Gulf are somewhat different from those of the Pacific Ocean where there are warmer waters at the end of the year, especially during yellowfin tuna spawning seasons. There is also high productivity characterized by the presence of significant biomass abundance of sardine or anchovy schools (Cisneros-Mata et al., 1995), which represents opportunities to establish the feeding and consequently the spawning grounds for eastern Pacific yellowfin tuna. Likewise, there is a trend of migratory movements through the Gulf of California by different groups of yellowfin tunas (Fink and Bayliff, 1970). These movements promote stock mixing and help to explain the wide polymorphism displayed in this sample, in contrast to the weak variation found in other samples from the coast and offshore regions. Further genetic research, including sequential temporal sampling of young fishes in order to ensure the presence of individu-

als that originated in discrete spawning grounds, should be undertaken to prove the presence of an independent unit inside the Gulf of California, which, if confirmed, might necessitate new stock management strategies.

Allele frequencies for *Gpi-F** locus found in the present study, apparently, correspond to those reported by Ward et al. (1994 and 1997). These authors reported a higher proportion of the allele *Gpi-F*75* (0.571) in the eastern Pacific region and a gradual decrement of the same allele toward the central (0.423) and western Pacific regions (0.330), where allele *Gpi-F*100* (0.650) had the higher proportion. In the present study, the highest frequencies for the allele *Gpi-F*75* corresponded to the region of the eastern Pacific, situated in the limits of the yellowfin tuna regulatory area (offshore region), and there was a slight decrease in frequencies towards the coastal area (Table 5). Furthermore, allele frequencies for the *Gpi-F*75* allele from the coastal locality, Colima (0.444), and the intermediate locality southeast, Clipperton Islands (0.409), have coincidences with those reported by Ward et al. (1994) for the collection Hawaii 92 (0.423) in the central Pacific region.

The similarities in the *Gpi-F** allelic frequencies between eastern (Colima and Clipperton) and central Pacific samples (Hawaii 92) might possibly be attributed to the extended migrations of yellowfin tuna in the eastern Pacific brought about by the strong influence of warm waters on tuna movements because of the increased depth of the thermocline layer in that area, which was reflected by a decrease in catches (Joseph and Miller, 1988; Wild, 1994) and which possibly led to the mixing of the eastern and central Pacific stocks.

The low number of RAPD loci analyzed and the uncertainty of fulfilling some assumptions, such as the genetic identity of each band needed for qualitative and quantitative interpretation of data in terms of allelic frequencies, do not allow us to consider our estimations of subdivision reliable with the RAPD method. Additionally, the lack of reliability of estimations associated with high sampling variances by using randomly collected fishery samples highlights the need to design more efficient spatial and temporal sampling strategies in local and wide areas, as well as the need for alternative hypervariable markers to assess the divergence patterns observed in highly migratory species.

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