

Analyses of Nuclear *ldhA* Gene and mtDNA Control Region Sequences of Atlantic Northern Bluefin Tuna Populations

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Abstract: There has been considerable debate about whether the Atlantic northern bluefin tuna exist as a single panmictic unit. We have addressed this issue by examining both mitochondrial DNA control region nucleotide sequences and nuclear gene *ldhA* allele frequencies in replicate size or year class samples of northern bluefin tuna from the Mediterranean Sea and the northwestern Atlantic Ocean. Pairwise comparisons of multiple year class samples from the 2 regions provided no evidence for population subdivision. Similarly, analyses of molecular variance of both mitochondrial and *ldhA* data revealed no significant differences among or between samples from the 2 regions. These results demonstrate the importance of analyzing multiple year classes and large sample sizes to obtain accurate estimates when using allele frequencies to characterize a population. It is important to note that the absence of genetic evidence for population substructure does not unilaterally constitute evidence of a single panmictic population, as genetic differentiation can be prevented by large population sizes and by migration.

Key words: Atlantic bluefin tuna, population genetics, control region, mtDNA, *ldhA* gene.

INTRODUCTION

The International Commission for the Conservation of Atlantic Tunas (ICCAT) currently manages the Atlantic northern bluefin tuna (BFT) as 2 stocks, one from the western Atlantic Ocean and the other from the eastern Atlantic Ocean and the Mediterranean Sea, with the division at longitude 45° W (ICCAT, 2001). The 2 manage-

ment units are based partly on the existence of only 2 known spawning areas, the Mediterranean Sea and the Gulf of Mexico. For management purposes it is assumed that fisheries west of longitude 45° W predominately catch “western stock” and that fisheries east of longitude 45° W catch predominately “eastern stock.” Because of the economic value of the fishery, the stock issue is exceedingly contentious (Dean, 1997).

A review of extensive conventional tagging data has suggested that trans-Atlantic migration may be occurring at levels sufficiently high that the BFT could be considered a single population unit for management purposes (National

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Research Council, 1994). Tagging studies using satellite technology have confirmed the historical data and demonstrated that tuna tagged near the U.S. coast frequently cross into the eastern management zone (Block et al., 1998, 2001; Lutcavage et al., 1999). However, even though the new technology provides a wealth of information about the movement of individual fish, it does not provide information about where individual fish spawn.

The tools of population genetics provide another method for analyzing bluefin tuna stock structure (Dean and Woodley, 1994). Since population subdivision can result in the genetic differentiation of populations isolated by genetic drift and selection (Nei, 1987), the primary hypothesis we tested was that BFT from the western Atlantic and the Mediterranean represent samples of a single, genetically homogenous population (Dean and Woodley, 1994). Analyses comparing mitochondrial DNA control region nucleotide sequences from 140 BFT tuna captured in the western Atlantic and the Mediterranean demonstrated considerable heterogeneity (Alvarado Bremer et al., 1999b). Preliminary evidence for population subdivision was obtained by Broughton and Gold (1997), who used microsatellite assays to measure genetic variation in a small number of BFT. In this report we analyze both the mtDNA control region and the nuclear *ldhA* locus in replicate year class samples representing 245 individual BFT from the northwest Atlantic Ocean and the Mediterranean Sea. In contrast to previous genetic analyses based on small sample sizes, neither of these data sets provided evidence for population subdivision of BFT.

MATERIALS AND METHODS

Samples of the 1993 year class ($n = 37$) were obtained from collections made in 1993 and 1994, and samples of the 1998 year class ($n = 37$) were collected as young of the year in 1998. In addition, samples of BFT were obtained from the western Mediterranean Sea in 1997 and 1998 and divided into year classes based on the ICCAT estimated age length table (Turner, 1994). Since there is significant variation with age (Karakulak and Oray, 2001), samples with lengths representative of the 1990 year class ($n = 30$) and 1992 year class ($n = 32$) were chosen to minimize year class overlap. To obtain temporally distinct samples from the northwestern Atlantic Ocean, we divided a sample of BFT collected in 1994 into 2 size classes of approximately equal numbers based on an estimated age at length (Turner, 1994). The smaller size

class, 127 to 190 cm, corresponded to fish 5 to 9 years old ($n = 39$), and the larger size class, 197 to 277 cm ($n = 35$), corresponded to fish that were more than 10 years old.

Total DNA was extracted and polymerase chain reaction (PCR) amplifications of the mitochondrial D-loop region were performed as described by Alvarado Bremer et al. (1996). PCR products were purified for nucleotide sequence analysis using the QIAquick PCR Purification Kit (Qiagen Inc., Chatsworth, Calif.), and 335 bases of nucleotide sequence were obtained using a LI-COR (Lincoln, Neb.) automated DNA sequencer. Intron 6 of the *ldhA* gene was amplified as described by Quattro and Jones (1999). Nucleotide sequence analyses of the amplified products identified 3 alleles among northern bluefin tuna and showed that these 3 alleles could be discriminated by cleavage with the restriction enzyme *AseI* and *MwoI*. Therefore, restriction assays employing these enzymes were used for the population analyses.

For the mtDNA nucleotide sequence data, the total number of distinct haplotypes was determined from phylogenetic analyses using test Version 4.0b3a of PAUP, written by David L. Swofford (2000). Phylogenetic trees were constructed using neighbor-joining (NJ) analyses (Saitou and Nei, 1987) and Tamura-Nei's distances. In cases of missing data or insertions and deletions (indels), the sites were ignored in the affected pairwise comparisons. A yellowfin tuna control region nucleotide sequence (Alvarado Bremer et al., 1997) was used as the outgroup. Nucleotide and haplotypic diversities were calculated as defined by Nei (1987) using ARLEQUIN (Schneider et al., 1997).

For both sets of data, the level of genetic population differentiation was estimated using a nested analysis of molecular variance (AMOVA; Excoffier et al., 1992) with Tamura-Nei distances as implemented in ARLEQUIN. Samples were nested by year class and sample location. In addition, because the mtDNA data were nucleotide sequences, the sample data were analyzed for geographic structure using an alternative approach to the hierarchical analysis of nucleotide diversity as described by Holsinger and Mason-Gamer (1996).

RESULTS

Mitochondrial DNA D-Loop Analyses

As observed previously (Alvarado Bremer et al., 1997), we found considerable genetic variation among the BFT

Table 1. Sample Diversity with mtDNA Control Region Nucleotide Sequence Data

Sample	<i>N</i>	Number of Haplotypes	Haplotypic diversity	Number of polymorphic sites	Nucleotide diversity
Med 1990	31	29	0.996	86	0.048
Med 1992	32	28	0.990	60	0.026
Med 1993	37	32	0.981	75	0.035
Med 1998	38	31	0.989	84	0.043
At1 large	34	30	0.991	68	0.029
At1 small	38	28	0.983	51	0.023

Table 2. Analysis of Molecular Variance of mtDNA Control Region Nucleotide Sequence Data

Source of variation	<i>df</i>	Sum of squares	Variance components	Percentage of variation
Among groups	1	5.64	-0.0165	-0.29
Among populations, within groups	4	28.68	0.0442	0.78
Within populations	205	1153.03	5.6245	99.51
Total	210	1187.34	5.6522	

Table 3. Matrix of Probabilities from Pairwise Comparisons of F_{st} Values for mtDNA Control Region Nucleotide Sequence Data

Sample	At1 large	At1 small	Med 1990	Med 1992	Med 1993
At1 small	.547				
Med 1990	.290	.215			
Med 1992	.137	.148	.365		
Med 1993	.943	.212	.926	.097	
Med 1998	.137	.319	.504	.018	.209

mtDNA D-loop nucleotide sequences, with sample nucleotide diversities of 2.3% to 4.8% (Table 1). Almost all individuals had unique haplotypes, and haplotype diversities ranged from 0.98 to 0.99. An AMOVA demonstrated that more than 99% of the variance occurred within year classes, and no significant variation could be attributed to variation between ocean basins or among samples within an ocean basin (Table 2). Similarly, analyses using the method of Holsinger and Mason-Gamer (1996) failed to find significant differences among the samples. In pairwise comparisons, no statistically significant differences were found among the 6 samples (Table 3). Thus analyses of mtDNA data failed to provide evidence for population subdivision between Mediterranean (eastern) and western Atlantic BFT.

Nuclear Gene *ldhA* Analyses

Since mtDNA provides information only about maternal inheritance, we also analyzed intron 6 of the nuclear lactate dehydrogenase gene *ldhA* (Quattro and Jones, 1999). Re-

Table 4. Sample Allele Frequencies at the *ldhA* Locus

Sample	Allele		
	A12	A13	A14
Med 1990	.44	.31	.26
Med 1992	.41	.19	.40
Med 1998	.09	.28	.63
At1 large	.40	.24	.36
At1 small	.45	.27	.28

strictions fragment length polymorphism assays were used to determine the allelic composition of three Mediterranean and two Atlantic sample sets (Table 4). When an AMOVA analysis was performed, none of the variance could be attributed to the between-group comparison, but 7.7% of the variance was due to within-group differences (Table 5). When pairwise comparisons were performed, the 1998 year class sample was significantly different from the other 4 samples (Table 6). No other significant differences were observed.

Table 5. Analysis of Molecular Variance of *ldhA* Allele Frequency Data

Source of variation	<i>df</i>	Sum of squares	Variance components	Percentage of variation
Among groups	1	1.40	-0.0036	-1.09
Among populations, within groups	3	6.11	0.0254	7.67
Within populations	333	103.23	0.3100	93.42
Total	337	110.74	0.3318	

Table 6. Matrix of Probabilities from Pairwise Comparisons of F_{st} values for *ldhA* Allele Frequency Data

Sample	At1 small	At1 large	Med 1998	Med 1990
At1 large	.67			
Med 1998	.00	.00		
Med 1990	.92	.47	.00	
Med 1992	.35	.82	.00	.21

DISCUSSION

BFT are presently thought to spawn only in the Gulf of Mexico and the Mediterranean Sea. The presence of 2 geographically distinct spawning areas has led to the hypothesis that there might be two subpopulations (stocks) of BFT. However, neither the mitochondrial nor the *ldhA* nuclear data presented here provide evidence for population subdivision of BFT. Genetic analyses of yellowfin tuna (Graves, 1998; B. Ely et al., manuscript in preparation; but see Ward et al., 1997), skipjack tuna (Graves et al., 1984; B. Ely et al., manuscript in preparation), and southern bluefin tuna (Grewe et al., 1997) also have failed to find evidence for population substructure in highly migratory tunas. Nevertheless, failure to reject the null hypothesis of genetic homogeneity does not prove that it is true. Historically high population levels (Cau, 2000) or straying between populations (National Research Council, 1994) may have prevented genetic differences from accumulating.

The high level of haplotypic diversity observed in Atlantic BFT mtDNA would be consistent with historically high population levels and large effective population sizes that might prevent genetic differentiation. In contrast, similar levels of haplotypic diversity have been observed in swordfish mtDNA, and genetic differentiation is observed between Atlantic and Mediterranean populations of swordfish (Alvarado Bremer et al., 1999a). Both tagging studies (De la Serna and Alot, 1990) and genetic studies (Alvarado Bremer et al., 1999a) have demonstrated that Mediterranean swordfish migrate into the Atlantic, but that

the zone of mixing is confined to a small region close to the Straits of Gibraltar. Natal homing and low levels of straying prevent significant levels of genetic exchange between the two subpopulations of swordfish. This behavior contrasts markedly with the highly migratory behavior of BFT (Mather et al., 1974; National Research Council, 1994; Block et al., 1998; Lutcavage et al., 1999). Thus low, but significant, rates of migration may prevent population subdivision in BFT.

Both the mtDNA D-loop nucleotide sequence data and the *ldhA* allele frequency data provide examples of the importance of analyzing large sample sizes from multiple year classes to generate accurate assessments of allele frequency variation. Preliminary analyses of mtDNA D-loop nucleotide sequence data suggested differences in the distribution of haplotypes between northwestern Atlantic and Mediterranean population samples (Alvarado Bremer et al., 1999b). However, when the total sample size was increased and samples representing multiple year classes were examined, differences in haplotype distribution were no longer observed. Similarly, when the *ldhA* allele frequencies were compared, allele frequencies of the 1998 Mediterranean year class were different from those of the other samples. If the 1998 year class sample was the only Mediterranean sample analyzed, we might have concluded that significant allele frequency differences existed between Mediterranean and northwestern Atlantic BFT. Rather, the data demonstrate that it is possible for a single sample ($n = 37$) to have allele frequencies that are not representative of the general population. This result demonstrates the importance of sampling multiple year classes to avoid drawing erroneous conclusions from samples with atypical allele frequencies.

The classical and current tagging studies described earlier clearly show that trans-Atlantic movement of BFT does occur. If these migrations result in genetic exchange between 2 subpopulations of BFT, they could prevent genetic differentiation from occurring. Alternatively, the tagging data could represent movements within a single population. Unfortunately, the currently available tagging data cannot discriminate between the 2 stock and 1 stock

hypotheses because tagging studies are not linked directly to spawning events. Thus further studies using a variety of genetic and nongenetic approaches, including tagging and reproductive ecology studies, are needed to determine whether the BFT population is actually subdivided, and whether there is sufficient movement throughout the Atlantic to justify managing the fishery as a single stock.

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