Journal of Fish Biology (2005) **66,** 1545–1557
doi:10.1111/j.1095-8649.2005.00705.x, available online at http://www.blackwell-synergy.com

Genetic isolation between Atlantic and Mediterranean albacore populations inferred from mitochondrial and nuclear DNA markers

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(Received 7 June 2004, Accepted 17 January 2005)

Genetic population structure of Atlantic and Mediterranean albacore *Thunnus alalunga* was investigated using nucleotide sequence variations of the glucose-6-phosphate dehydrogenase gene intron (*G6PD*) and the mitochondrial DNA (mtDNA) D-loop region (*Dloop*). Restriction analysis using *Ase* I digestion detected two major restriction types (*A* and *B*) at the *Dloop* locus with strong frequency differences between Atlantic and Mediterranean samples. Thirty-six individuals of 100 Mediterranean albacore were of the *B* type whereas no *B* type individuals were found in the Atlantic samples ($n = 102$). Phylogenetic analysis using nucleotide sequence data of the *Dloop* locus indicated that the *B* type lineage recently arose from the ancestral *A* lineage in the Mediterranean Sea and has not dispersed into the Atlantic Ocean. The frequencies of two alleles (*L* and *S*) at the *G6PD* locus were significantly different between the samples from the Atlantic ($L = 0{\cdot}495$) and the Mediterranean ($L = 0{\cdot}725$), but no significant heterogeneity was observed between mtDNA-*A* and -*B* types of the Mediterranean sample. These molecular data indicate that gene flow between the Atlantic and Mediterranean albacore populations have been considerably restricted and strongly suggest these populations should continue to be treated as two distinct management units.

Key words: albacore; Atlantic and Mediterranean; G6PD; genetic stock structure; mtDNA.

INTRODUCTION

The albacore *Thunnus alalunga* (Bonnaterre) is a highly migratory cosmopolitan tuna distributed throughout tropical and temperate areas of all oceans including the Mediterranean Sea (Collette & Nauen, 1983). The populations of these ocean basins have been managed as different stocks based on the available evidence for geographic separation and distinct spawning areas and seasons, despite a lack of understanding regarding the extent and magnitude of migration between ocean basins. Populations in the northern and southern hemispheres of the Atlantic and Pacific Oceans, respectively, are also separately managed. The spawning grounds appear to be discontinuous between the hemispheres with

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opposite seasonal peaks. Hooking rates also differ between northern and southern populations, and catch in the equatorial area is very low (Otsu & Uchida, 1959; Beardsley, 1969; Nakamura, 1969; Suzuki *et al.*, 1977; Murray, 1994). According to these ecological and fisheries characteristics, Nakamura (1969) and Lewis (1990) considered albacore migration across the equator to be negligible.

Recent molecular population genetic analyses have also supported the stock structures and management units described above. Using PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis on the albacore mitochondrial DNA (mtDNA), Chow & Ushiama (1995) found considerably different haplotype distributions between Atlantic and Pacific samples. Takagi *et al.* (2001), using highly polymorphic nuclear microsatellites, detected genetic heterogeneity not only between Atlantic and Pacific samples but also between northern and southern samples within each ocean.

There appears to be a distinct spawning ground for albacore in the Mediterranean Sea (Collette & Nauen, 1983), and the growth rates of Mediterranean and Atlantic albacore are reported to be different (Megalofonou, 2000). Furthermore, tag-recovery investigations from 1968 to 1999 also indicated restricted movement of albacore between these ocean basins (Arrizabalaga *et al.*, 2002). Despite this ecological evidence, molecular analyses have detected no (Viñas *et al.*, 1999; Pujolar *et al.*, 2003) or little genetic differentiation between Atlantic and Mediterranean albacore samples (Viñas *et al.*, 2004). Highly variable DNA markers such as microsatellites seem promising, but they usually require large numbers of individuals for statistical robustness. Less variable but still practical DNA markers have been surveyed, and simple but diagnostic variations found at the nuclear calmodulin gene locus (*CaM*) separating the North and South Atlantic populations of the swordfish *Xiphias gladius* L. (Chow & Takeyama, 2000) and a coding region of mtDNA characterizing Atlantic and Indo-Pacific populations of the bigeye tuna *Thunnus obesus* (Lowe), (Chow *et al.*, 2000). These findings indicate that the appearance of genetic population structure depends on contemporary and historical events, but the subsequent detection of this structure would depend on sample design and the characteristics of the molecular marker.

Recently, Chow & Nakadate (2004) designed semi-universal PCR primers to amplify the fourth intron of the fish glucose-6-phosphate dehydrogenase (G6PD) gene and found length variation in the amplified fragments from an albacore sample. Using nucleotide sequence analysis they revealed a large insertion or deletion of a nucleotide block (110 bp) in the introns between the two alleles (designated as *A* and *B*), which made genotype scoring using agarose gel electrophoresis easy and reliable. This polymorphic nuclear gene marker was used in the present study to compare Atlantic and Mediterranean samples of albacore. Nucleotide sequencing and RFLP analyses were also conducted on the hyper-variable mtDNA D-loop region.

MATERIALS AND METHODS

FISH SAMPLES

The three local samples of albacore used in this study were from the north-east Atlantic (NEA)(Bay of Biscay)($n = 66$), the south-west Atlantic (SWA)(off Brazil)($n = 77$) and the

Mediterranean Sea (MED)($n = 104$)(Fig. 1). The North and South Atlantic samples were collected in 1993 by the Institute de Recherche pour le Developpement (IRD) and in 1994 by the Fisheries Institute of Sao Paulo (IP-SP), respectively, and these were the same samples used by Chow & Ushiama (1995). The Mediterranean sample consisted of three sub-samples (MEDL, MED93 and MED03). A larval sample (MEDL) was collected in a plankton survey conducted by the RV Shoyo-Maru (Fishery Agency of Japan) in 1994. The frozen larvae were transferred to the laboratory where species identification was carried out using morphological and molecular analyses (Kohno *et al*., 1982; Chow & Inoue, 1993; Chow & Kishino, 1995; Takeyama *et al*., 2001; Chow *et al*., 2003). Other Mediterranean samples were young adults and adults caught in the Aegean and Ionian Seas in 1993 (MED93) (mean fork length, L_F, = 70 cm) and those caught in the Ionian Sea in 2003 (MED03) (mean $L_F = 78$ cm).

DNA ANALYSIS

PCR primers for amplifying a segment between the third region of exon 4 and the fifth region of exon 5 containing the entire intron 4 of the G6PD gene are 5 -GAGCAGACG-TATTTTGTGGG-3 (G6PDex4F) and 5 -GCCAGGTAGAAGAGGCGGTT-3 (G6PDex5R)(Chow & Nakadate, 2004). Primer sequences to amplify the hyper-variable left domain of the mtDNA D-loop are 5 -GTACATATATGTAATTACACC-3 (DLStunaF1) and 5 -CTTCAATAACCGTATGCATT-3 (DLStunaR1). The same amplification conditions were applied to these two gene segments. Amplification was carried out using an initial denaturation at 95° C for 2 min, followed by 30 cycles of amplification (denaturation at 95° C for 0·5 min, annealing at 55° C for 1 min and extension at 72° C for 2 min) with a final extension at 72° C for 10 min. PCR products from the G6PD gene were directly subjected to agarose gel electrophoresis (2–2·5%) to observe variation in

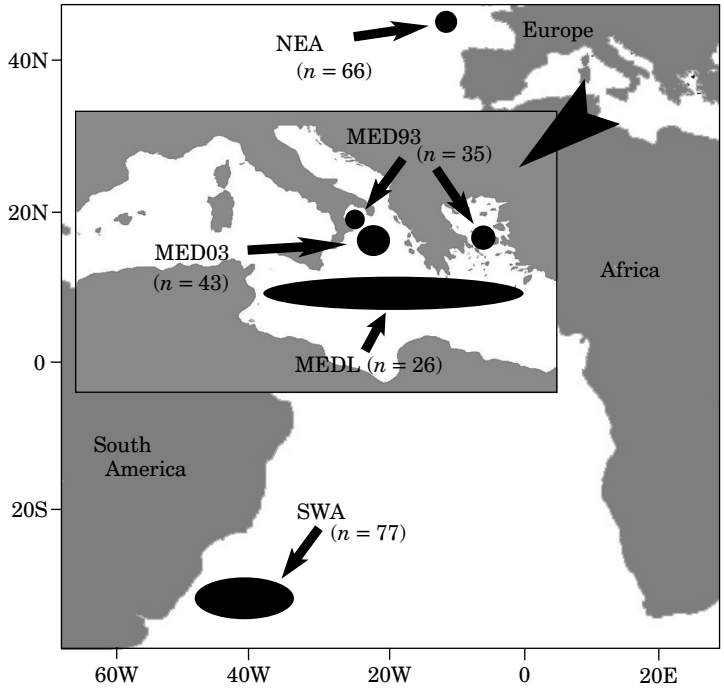

FIG. 1. Map showing catch localities and number of individuals of albacore samples used in this study. The Mediterranean sample consisted of three sub-samples: MEDL, MED93 and MED03. NEA, north-east Atlantic; SWA, south-west Atlantic.

amplified fragment length and to score genotypes as described in Chow & Nakadate (2004). Since preliminary RFLP surveys for the *Dloop* locus indicated *Ase* I digestion yielded heterogeneous distributions of the restriction types among samples, the restriction assay was performed on all samples.

Direct nucleotide sequence analysis for the D-loop fragment was performed on a total of 48 albacore ($n = 14$ in NEA, 18 in SWA and 16 in MED), including arbitrarily selected individuals and those having specific restriction types, from each of the three locations. Two Pacific northern bluefin tuna *Thunnus thynnus orientalis* (Temminck & Schlegel) were also included as an out-group species. The PCR products were treated with ExoSAP-IT (Amersham Biosciences) to prepare the DNA template. Automated sequences were generated on an automated sequencer (ABI Prism310) using the ABI Big-dye Ready Reaction kit (Applied Biosystems) following the standard cycle sequencing protocol.

DATA ANALYSIS

Spatial genetic variation was quantified by estimating analogs of Wright's F_{ST} (Φ_{ST} for mtDNA haplotype and sequence data and F_{ST} for allele frequency data of *G6PD*) using analysis of the molecular variance (AMOVA) (Excoffier *et al*., 1992). In both cases, the estimates were computed for all five population samples as well as for the two geographic samples (Atlantic and Mediterranean) using the programme ARLEQUIN vers. 2·0 (Schneider *et al*., 2000). The significance level of F_{ST} analogs was tested using 10000 permutations, and the Bonferroni (Rice, 1989) and Holm (Holm, 1979) methods were used to correct for multiple comparisons.

Nucleotide sequence data were imported into MEGA (Kumar *et al*., 2001) to calculate nucleotide diversity based on Kimura's two parameter distance (K2P) and to construct neighbour-joining (NJ) and maximum parsimony (MP) phylogenetic trees. The data were also imported into PHYML (Guindon & Gascuel, 2003) for maximum likelihood analysis (ML). In ML analysis, the Hasegawa-Kishino-Yano model (Hasegawa *et al*., 1985) with gamma correction (HKY85 + G) was selected as the best fit by the MODELTEST procedure (Posada & Crandall, 1998). During the exploration of the ML topology, all parameters of the nucleotide substitution model and the gamma shape parameter were simultaneously estimated and adjusted. In order to evaluate the reliability of each node, bootstrap analysis was carried out by PHYML using the 1000 resampled data sets produced by SEQBOOT from the PHYLIP package (Felsenstein, 1993). For comparison to the NJ, MP and ML analyses, network reconstruction (Posada & Crandall, 2001) was used to infer phylogenetic relationships *via* a median-joining (MJ) network drawn with NETWORK 3.1.1.1 (Bandelt *et al*., 1999).

RESULTS

GENOTYPE ANALYSIS

Genotype and allele frequencies at *G6PD* and *Dloop* loci are presented in Table 1. Fragment length variation at the *G6PD* locus observed in the present study was identical to that reported by Chow & Nakadate (2004), and the two alleles (*A* and *B*) identified in that study are re-designated here as *L* (longer) and *S* (shorter), respectively, to avoid confusion. Genotype proportions in all samples were in accordance with Hardy-Weinberg expectations. Expected heterozygosity (*He*) ranged from 0·327 (MEDL) to 0·498 (SWA), and the frequency of the *L* allele ranged from 0·439 (NEA) to 0·800 (MEDL). The size of amplified fragments from the mtDNA D-loop region was estimated to be *c*. 310 bp, and no apparent size variation on the agarose gel was observed among a total of 202 individuals examined. Of the four genotypes (designated as *A*, *B*, *C* and *D*

TABLE I. Genotype and allele frequencies at *G6PD* and *Dloop* loci in five albacore samples

	Mediterranean			Atlantic	
	MED93	MEDL	MED03	NEA	SWA
G6PD					
LL	19	17	13	11	22
LS	12	6	20	36	41
SS	3	2	3	19	14
Total	34	25	36	66	77
L	0·735	0·800	0·639	0·439	0·552
S	0·265	0·200	0·361	0·561	0·448
Ho	0·358	0·245	0·563	0·550	0·536
He	0·395	0·327	0·468	0·496	0·498
Dloop					
A	26	14	23	58	43
B	5	12	19	0	0
C	0	0	0	0	1
D	0	0	1	0	0
Total	31	26	43	58	44
h	0·28	0·517	0·53	0	0·045

h, haplotype diversity.

types) detected by the *Ase* I digestion (Fig. 2), *C* and *D* types were rare, occurring in one individual each. The Mediterranean samples were polymorphic, with the frequency of the *B* type ranging from 16·1 to 46·2% as compared to 0·0% for the Atlantic samples.

AMOVA conducted for all five populations (*i.e.* three Mediterranean and two Atlantic) in a single group indicated statistically significant genetic heterogeneity in *Dloop* ($\Phi_{ST}=0{\cdot}297$, $P<0{\cdot}001$) and *G6PD* ($F_{ST}=0{\cdot}068$, $P<0{\cdot}001$) loci. When Mediterranean (one group with three populations) and Atlantic (one group with two populations) analyses were conducted separately, significant differences were observed for the *Dloop* samples in the Mediterranean only ($\Phi_{ST}=0{\cdot}082$, $P=0{\cdot}019$). No significant differences were observed at *G6PD* locus for the Mediterranean samples ($F_{ST}=0{\cdot}016$, $P=0{\cdot}136$) nor at either loci in the Atlantic samples ($\Phi_{ST}=0{\cdot}006$, $P=0{\cdot}434$ for *Dloop* and $F_{ST}=0{\cdot}018$, $P=0{\cdot}073$ for *G6PD*). Given the ambiguous AMOVA results for the Mediterranean, a complete set of pairwise comparison tests for the F_{ST} and Φ_{ST} values from the five populations was computed. As shown in Table II, no significant differences in haplotype and allele frequencies at either the *G6PD* and *Dloop* locus was observed between three Mediterranean samples after Bonferroni and Holm corrections. Furthermore, no significant allele frequency difference was observed at the *G6PD* locus between mtDNA-*A* ($n=58$; *D* type included) and -*B* ($n=33$) types of the Mediterranean sample ($F_{ST}=-0{\cdot}009$, $P=0{\cdot}611$). On the basis of these results, the Mediterranean samples were pooled and tested against the Atlantic samples in an AMOVA with two groups (i.e. Mediterranean and

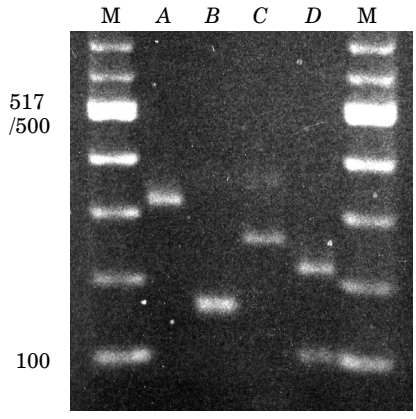

FIG. 2. Four restriction profiles (*A* to *D*) of the D-loop fragments of albacore obtained by *Ase* I digestion. M, molecular size marker (100 bp ladder).

Atlantic). Results showed highly significant differences ($P < 0{\cdot}001$) for both gene loci ($\Phi_{ST} = 0{\cdot}337$, $F_{ST} = 0{\cdot}087$).

NUCLEOTIDE SEQUENCE ANALYSIS OF THE MTDNA DLOOP FRAGMENT

Among the 13 larvae and three adults selected from the Mediterranean sample, seven were *A* type, eight were *B* type and one was *D* type in the *Ase* I restriction assay. Fourteen and 18 individuals were chosen from the North and South Atlantic samples, respectively, in which one South Atlantic individual was *C* type and the remainder were *A* type. Sequence alignment of these individuals with Pacific northern bluefin tuna (PNB) is shown in Fig. 3. The aligned fragments consisted of 267 to 269 nucleotides, and 87 variable sites were observed among the 48 albacore sequences. In contrast to the restriction assay, haplotype diversity based on nucleotide sequence was significantly higher in the Atlantic samples (mean $\pm$ S.D. $h = 1{\cdot}000 \pm 0{\cdot}008$) than in the Mediterranean samples ($h = 0{\cdot}942 \pm 0{\cdot}048$). All Atlantic albacore ($n = 32$) had different sequences to one another. In contrast, two individuals shared one sequence

TABLE II. Pair-wise estimates of F_{ST} analogs at *G6PD* (above diagonal) and *Dloop* (below diagonal) loci between five albacore samples

	MED93	MEDL	MED03	NEA	SWA
MED93		−0·006	0·007	0·151*	0·058
MEDL	0·165		0·044	0·216*	0·108*
MED03	0·133	−0·030		0·066	0·005
NEA	0·197*	0·570*	0·450*		0·018
SWA	0·138	0·486*	0·391*	0·006	

*Significant after Bonferroni and Holm corrections.

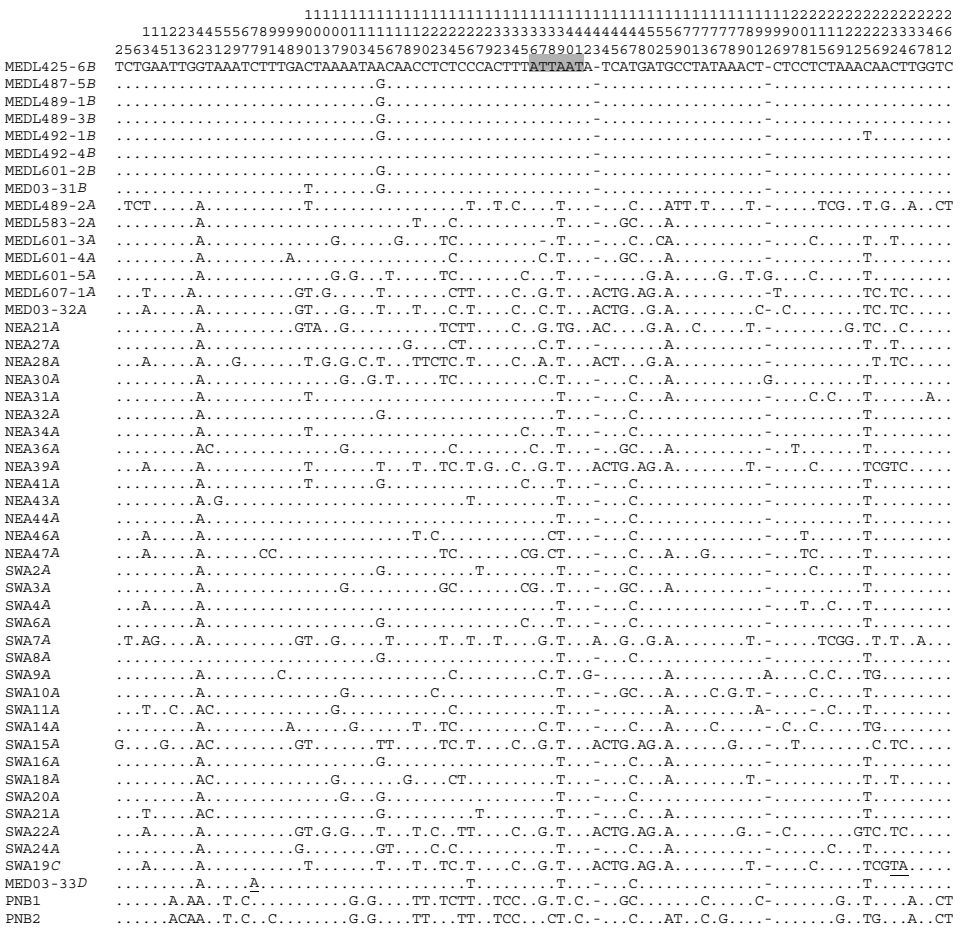

FIG. 3. Polymorphic nucleotide sites in the *Dloop* locus from 48 albacore and two Pacific northern bluefin tunas (PNB). Restriction types (*A* to *D*) in the albacore are noted at the end of each specimen number. Identity with the top reference sequence is indicated by ·, and gaps are denoted by -. The diagnostic *Ase* I restriction site separating *A* and *B* types is shaded, and nucleotide substitutions causing other *Ase* I site gains for *C* and *D* types are underlined. Representative sequences of four restriction types (NEA21, MEDL425–6, SWA19 and MED03–33) are available in the DNA Data Bank of Japan (DDBJ) under accession numbers AB181140 to AB181143.

and four individuals shared another sequence in the Mediterranean sample ($n = 16$) and these six individuals were the *B* type.

Ase I site loss or gain responsible for separating *A* and *B* types was observed at 136 to 141 nt (Fig. 3, shaded), and none of 32 Atlantic albacore individuals was observed to possess this *Ase* I site. This *Ase* I site was not observed in *C* and *D* types, but other *Ase* I sites caused by nucleotide substitutions at 232 and 234 nt and at 77 nt, respectively (Fig. 3, underlined) were observed in these two types. Mean ± S.D. nucleotide diversity (π) based on Kimura's two parameter model (K2P) for the entire albacore sample was $0{\cdot}0561 \pm 0{\cdot}0076$, and π values within geographic areas were $0{\cdot}0498 \pm 0{\cdot}0078$ (MED), $0{\cdot}0547 \pm 0{\cdot}0082$ (NEA) and $0{\cdot}0608 \pm 0{\cdot}0087$ (SWA). For *B* type individuals, π was $0{\cdot}0035 \pm 0{\cdot}0020$

($n=8$), significantly smaller than values for the *A* type in the Mediterranean ($0{\cdot}0780 \pm 0{\cdot}0109$)($n=7$, *D* type not included) and the North and South ($0{\cdot}0577 \pm 0{\cdot}0081$)($n=17$, *C* type not included) Atlantic samples ($P<0{\cdot}001$). Although π for the *A* type of Mediterranean albacore was the highest, no significant difference was observed among *A* type samples from three ocean basins ($P>0{\cdot}1$). Thus, the low nucleotide diversity and repeated haplotypes within the *B* type appeared to be responsible for the lower diversity of the Mediterranean sample as compared to the Atlantic samples. AMOVA revealed slight but significant heterogeneity between Atlantic (NEA and SWA pooled) and Mediterranean samples ($\Phi_{ST}=0{\cdot}041$, $P=0{\cdot}046$).

Median-Joining (MJ) and NJ trees are shown in Fig. 4. Although all tree topologies supported two separated phylogroups (designated I and II), no apparent phylogeographic assignment of mtDNA lineages was observed. A robustly supported small cluster consisting only of the *B* type was observed in the phylogroup II (Fig. 4, asterisk in NJ and triangles in MJ). In these trees, the restriction types did not correspond to the phylogenetic implications, and the *B* type appeared to be a sub-type derived from the *A* type. The absence of *B* type individuals in the Atlantic and the low sequence divergence among *B* type individuals suggest that the mtDNA lineage having gained a novel *Ase* I site arose recently in the Mediterranean Sea and that the *B* type lineage has not dispersed into the Atlantic Ocean.

DISCUSSION

Viñas *et al.* (1999) compared Atlantic and Mediterranean albacore samples using nucleotide sequence analysis on the hyper variable left domain of mtDNA D-loop region, but they failed to detect significant differences between samples from the two locations. The small sample size (16 from the Atlantic and eight from the Mediterranean) and the use of only phylogenetic analysis may be responsible for not detecting genetic heterogeneity between the samples from these ocean basins. The present phylogenetic analysis using twice the number of samples ($n=48$) also did not greatly increase the resolving power for stock structure. Recently, Viñas *et al.* (2004) analysed *D-loop* sequences of much larger numbers of albacore (54 Atlantic, 50 Mediterranean and 30 Pacific). Haplotypes from all sampled regions, however were interspersed throughout the phylogenetic tree with no geographic association as observed in the present study. A slight but significant genetic structuring between Atlantic and Mediterranean samples was only detected in the analysis based on AMOVA (Viñas *et al.*, 2004), but much better resolution was obtained by the present RFLP analysis. Similar findings have been observed in other highly migrating fish species such as bigeye tuna and swordfish. In these species, simple RFLP analysis resolved genetic stock structuring within and between ocean basins (Kotoulas *et al.*, 1995; Alvarado Bremer *et al.*, 1996, 1998; Chow *et al.*, 1997; Chow & Takeyama, 2000; Chow *et al.*, 2000), even though RFLP markers were capable of detecting a much smaller number of genotypes than nucleotide sequence and microsatellite analyses. Thus, these results suggest that the level of genetic variability does not correspond to resolving power for clarifying

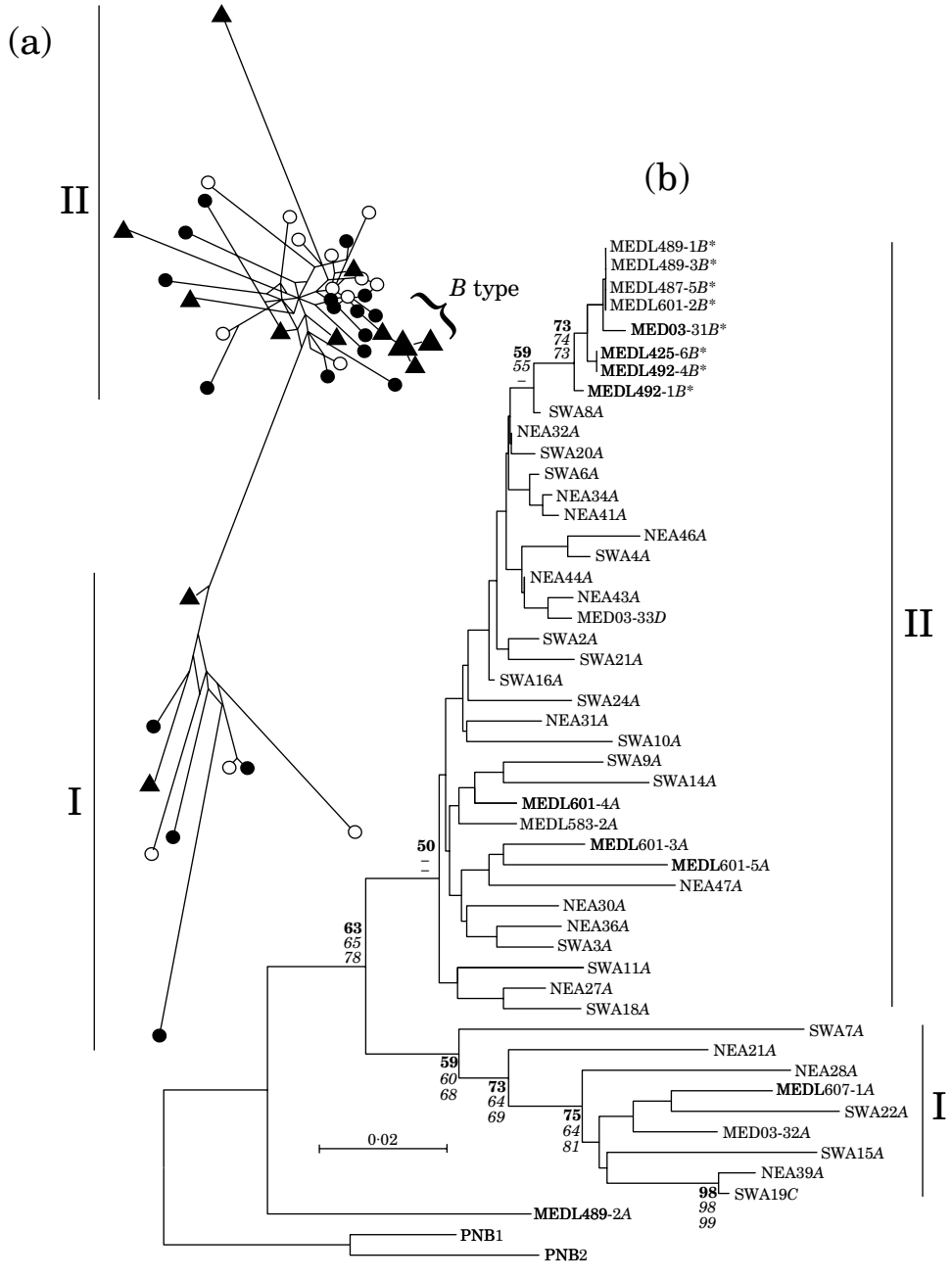

FIG. 4. (a) Median-joining network (MJ) and (b) neighbour-joining tree drawn using *Dloop* sequence data. Numbers above branches of NJ tree indicate bootstrap values from NJ analysis (in bold), parsimony analysis (MP) (in italics), and maximum likelihood analysis (ML) (normal text). The hyphens under the bootstrap value for the NJ method indicate that the bootstrap values using MP or ML methods are $< 50\%$. The geographic origin of each haplotype in the MJ network is annotated as Mediterranean (▲), North Atlantic (○) and South Atlantic (●). All tree topologies supported two separated phylogroups (designated I and II).

genetic relationships among sub-populations with low levels of gene flow over a large geographical scale.

The magnitude of differences in mtDNA genotype distributions between Atlantic and Mediterranean albacore populations observed in this study are similar to those between Atlantic and Indo-Pacific bigeye tuna populations and between Atlantic and Mediterranean swordfish populations. Of two genotypes (α and β) detected by RFLP analysis in the mtDNA ATPase-COIII gene region of bigeye tuna, the α type occurred at a high frequency (73%) in the Atlantic but was nearly zero (0·5%) in the Indo-Pacific (Chow *et al.*, 2000), clearly indicating no gene flow and migration from the Atlantic to the Indo-Pacific. Chow *et al.* (1997) and Chow & Takeyama (2000) observed seven genotypes by *Rsa* I restriction digestion in the mtDNA D-loop region of Atlantic swordfish but all Mediterranean individuals were type *C*, indicating no migration and gene flow from the Atlantic to the Mediterranean. Because of the haploid nature of mtDNA, however, all of these studies including the present investigation could not reject an alternative inference that gene flow or migration in the opposite direction is ongoing.

There is ample evidence indicating that the Mediterranean population of albacore is independent from the Atlantic population. Arena (1991) reported differences in morphometric features, such as a longer head and a shorter pectoral fin in Mediterranean individuals. The Mediterranean population displays separate spawning grounds (Piccinetti & Piccinetti-Manfrin, 1993; Piccinetti *et al.*, 1997) and seems to reach sexual maturity at a smaller size than the Atlantic population (62 *v.* 90 cm L_F) (SCRS, 1997). Comparison between Mediterranean and Atlantic growth variables, using scale or spine age estimates, indicates that Mediterranean individuals are significantly smaller than their Atlantic counterparts. It has been noted that the Mediterranean albacore do not reach the asymptotic L_F observed in albacore in the Atlantic Ocean and the mean L_F per age class for Mediterranean fish are smaller, especially for larger and presumably older fish (Megalofonou, 2000; Megalofonou *et al.*, 2003). Moreover, the comparison of means of growth in L_F (cm year^{-1}) observed in tagged albacore, at liberty for periods of 10 to 36 months in the Atlantic Ocean and Mediterranean Sea, showed a lower growth rate in the Mediterranean albacore (Ortiz de Zarate *et al.*, 1996; De Metrio *et al.*, 1997; Megalofonou, 2000). The L_F and mass relationship of Mediterranean albacore differs from that of Atlantic albacore and it was observed that at a given L_F, Atlantic albacore are heavier (Megalofonou, 1990, 2000). Tag and recapture data suggest low migration between the Atlantic Ocean and the Mediterranean Sea (De Metrio *et al.*, 1997; Ortiz de Zarate & Cort, 1998; Arrizabalaga *et al.*, 2002) and occasionally large individuals (supposedly of Atlantic origin) have been found within the Mediterranean Sea (Di Natale, 1990; G. De Metrio, pers. comm.), indicating that the Strait of Gibraltar is not a complete physical barrier for albacore.

Results obtained from the *G6PD* locus are the first nuclear DNA data revealing genetic heterogeneity between Atlantic and Mediterranean albacore. This corroborates mtDNA data supporting the existence of genetic isolation between Atlantic and Mediterranean albacore, and is associated with the ecological differences observed between the samples from these ocean basins. None

of these results, however, conclusively demonstrate complete isolation, and more data from the nuclear genome will be necessary to further investigate genetic relationships between these populations. The mtDNA sequence diversity among *A* type individuals from the Mediterranean Sea was the highest, suggesting that the Mediterranean albacore population has incorporated exogenous mDNA lineages and evolved independently over a long period. Considerably restricted uni-directional gene flow may be the most plausible hypothesis at present, although it is not known whether the gene flow is historical or ongoing.

Atlantic and Mediterranean albacore have been managed as distinct units by the International Commission for the Conservation of Atlantic Tunas (ICCAT), and Viñas *et al.* (2004), observing slight but significant genetic structuring between Atlantic and Mediterranean albacore samples, recommended that this management strategy continue. The present study further supports the management strategy but also offers a simple tool to investigate migration of Mediterranean albacore. The present study suggests that Mediterranean albacore do not penetrate deeply into the Atlantic Ocean, but the sampling localities in the Atlantic Ocean were too limited to resolve questions relating to the distance Mediterranean albacore may travel in the Atlantic Ocean. This question can be resolved by the analysis of samples from the adjacent waters of the Strait of Gibraltar, and progressively more distant waters of the central Atlantic. The RFLP marker of albacore mtDNA is simple and may be used to practically investigate the spatio-temporal dynamics of Mediterranean albacore migration around the Strait of Gibraltar area. Most swordfish individuals collected at Tarifa (just outside the Strait of Gibraltar) were shown to be of Mediterranean origin by mtDNA analysis (Chow & Takeyama, 2000), and this might also be the case with Mediterranean albacore. If so, the albacore catch in this area should be incorporated into the Mediterranean quota.

We thank S. Tsuji and crew of RV Shoyo Maru for assistance in sample collection and to J. R. Alvarado Bremer for allowing us to access their manuscript in press. Thanks are also due to G. De Metrio, Y. Takeuchi and anonymous reviewers for improving this manuscript. This work was supported in part by grants from the Japan Society for the Promotion of Science, the Ministry of Agriculture, Forestry and Fisheries of Japan, and a Grant-in-Aid for Scientific Research on Priority Areas (B) from the Ministry of Education, Science, Sports and Culture.

References

Alvarado Bremer, J. R., Mejuto, J., Greig, T. W. & Ely, B. (1996). Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial control region. *Journal of Experimental Marine Biology and Ecology* **197,** 295–310.

Alvarado Bremer, J. R., Stequert, B., Robertson, N. W. & Ely, B. (1998). Genetic evidence for inter-oceanic subdivision of bigeye tuna (*Thunnus obesus*) populations. *Marine Biology* **132,** 547–557.

Arena, P. (1991). Some biological features of albacore, *Thunnus alalunga* (Bonn.), in the Tyrrhenian Sea. *FAO Fisheries Report* **449,** 116–121.

Arrizabalaga, H., López-Rodas, V., Ortíz de Zárate, V., Costas, E. & González-Garcés, A. (2002). Study on the migrations and stock structure of albacore (*Thunnus alalunga*) from the Atlantic Ocean and the Mediterranean Sea based on conventional tag release-recapture experiences. *ICCAT Collective Volume of Scientific Papers* **54,** 1479–1494.

Bandelt, H-J., Forster, P. & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16,** 37–48.
Beardsley, F. L. (1969). Proposed migration of albacore (*Thunnus alalunga*) in the Atlantic Ocean. *Transactions of American Fisheries Society* **98,** 589–590.
Chow, S. & Inoue, S. (1993). Intra-and interspecific restriction fragment length polymorphism in mitochondrial genes of *Thunnus* tuna species. *Bulletin of the National Research Institute of Far Seas Fisheries* **30,** 207–225.
Chow, S. & Kishino, H. (1995). Phylogenetic relationships between tuna species of the genus *Thunnus* (Scombridae, Teleostei): inconsistent implications from morphology, nuclear and mitochondrial genomes. *Journal of Molecular Evolution* **41,** 741–748.
Chow, S. & Ushiama, H. (1995). Global population structure of albacore (*Thunnus alalunga*) inferred by RFLP analysis of the mitochondrial ATPase gene. *Marine Biology* **123,** 39–45.
Chow, S. & Takeyama, H. (2000). Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish. *Journal of Fish Biology* **56,** 1087–1098. doi: 10.1006/jfbi.2000.1229
Chow, S. & Nakadate, M. (2004). PCR primers for G6PD gene intron and characterization of intron length variation in albacore *Thunnus alalunga*. *Molecular Ecology Notes* **4,** 391–393.
Chow, S., Okamoto, H., Uozumi, Y., Takeuchi, Y. & Takeyama, H. (1997). Genetic stock structure of the swordfish (*Xiphias gladius*) inferred by PCR-RFLP analysis of the mitochondrial control region. *Marine Biology* **127,** 359–367.
Chow, S., Okamoto, H., Miyabe, N., Hiramatsu, K. & Barut, N. (2000). Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. *Molecular Ecology* **9,** 221–227.
Chow, S., Nohara, K., Tanabe, T., Itoh, T., Tsuji, S., Nishikawa, Y., Ueyanagi, S. & Uchikawa, K. (2003). Genetic and morphological identification of larval and small juvenile tunas (Pisces, Scombridae) caught by a mid-water trawl in the western Pacific. *Bulletin of the Fishery Research Agency* **8,** 1–14.
Collette, B. B. & Nauen, C. E. (1983). *FAO Species Catalogue. Scombrids of the World.* Rome: FAO.
De Metrio, G., Megalofonou, P., Cacucci, M., Sion, L., Ortiz de Zarate, V. & Acone, F. (1997). Results of tagging experiments on albacore (*Thunnus alalunga* Bonn.) in the Northern Ionian and Southern Adriatic Seas from 1990 to 1995. *ICCAT Collective Volume of Scientific Papers* **46,** 148–151.
Di Natale, A. (1990). Bluefin tuna (*Thunnus thynnus* L.) and albacore (*Thunnus alalunga* Bonn.) fishery in the southern Tyrrhenian Sea: 1985–1989 surveys. *ICCAT Collective Volume of Scientific Papers* **35,** 231–239.
Excoffier, L., Smouse, P. E. & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131,** 479–491.
Felsenstein, J. (1993). *PHYLIP (Phylogeny Inference Package), Version 3.572.* Seattle, WA: Department of Genetics, University of Washington (distributed by the author).
Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52,** 696–704.
Hasegawa, M., Kishino, H. & Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22,** 160–174.
Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6,** 65–70.
Kohno, H., Hoshino, T., Yasuda, F. & Taki, Y. (1982). Larval melanophore patterns of *Thunnus alalunga* and *T. thynnus* from the Mediterranean. *Japanese Journal of Ichthyology* **28,** 461–465.
Kotoulas, G., Magoulas, A., Tsimenides, N. & Zouros, E. (1995). Marked mitochondrial DNA differences between Mediterranean and Atlantic populations of the swordfish, *Xiphias gladius*. *Molecular Ecology* **4,** 473–481.
Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). *MEGA2: Molecular Evolutionary Genetics Analysis Software*. Tempe, AZ: Arizona State University.

Lewis, A. D. (1990). South Pacific albacore stock structure: a review of available information. In: *3rd South Pacific Albacore Research Workshop*, *Working Paper No. 5*, pp. 1–13. Noumea, New Caledonia: South Pacific Commission.
Megalofonou, P. (1990). Size distribution, length-weight relationships, age and sex of albacore, *Thunnus alalunga* Bonn., in the Aegean Sea. *ICCAT Collective Volume of Scientific Papers* **33,** 154–162.
Megalofonou, P. (2000). Age and growth of Mediterranean albacore. *Journal of Fish Biology* **57,** 700–715. doi: 10.1006/jfbi.2000.1345
Megalofonou, P., Yannopoulos, C. & Dean, J. M. (2003). The potential use of scales for estimating age and growth of Mediterranean albacore (*Thunnus alalunga*). *Journal of Applied Ichthyology* **19,** 189–194.
Murray, T. (1994). A review of the biology and fisheries for albacore, *Thunnus alalunga*, in the South Pacific Ocean. *FAO Technical Paper* **336,** 188–206.
Nakamura, H. (1969). *Tuna Distribution and Migration*. London: Fishing News Books.
Ortiz De Zarate, V., Megalofonou, P., De Metrio, G. & Rodriguez-Cabello, C. (1996). Preliminary age validation results from tagged-recaptured fluorochrome labelled albacore in northeast Atlantic. *ICCAT Collective Volume of Scientific Papers* **43,** 331–338.
Ortiz de Zarate, V. & Cort, J. L. (1998). Albacore (*Thunnus alalunga*, Bonnaterre) stock structure in the Atlantic Ocean, as inferred from distribution and migration patterns. *ICCAT Collective Volume of Scientific Papers* **50,** 251–260.
Otsu, T. & Uchida, R. N. (1959). Sexual maturity and spawning of albacore in the Pacific Ocean. *Fishery Bulletin US* **59,** 287–305.
Piccinetti, C. & Piccinetti-Manfrin, G. (1993). Distribution des larves de thonides en Mediterranée. *ICCAT Collective Volume of Scientific Papers* **40,** 164–172.
Piccinetti, C., Piccinetti-Manfrin, G. & Soro, S. (1997). Results of a research survey on tuna larvae in the Mediterranean. *ICCAT Collective Volume of Scientific Papers* **46,** 207–214.
Posada, D. & Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14,** 817–818.
Posada, D. & Crandall, K. A. (2001). Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution* **16,** 37–45.
Pujolar, J. M., Roldán, M. I. & Pla, C. (2003). Genetic analysis of tuna populations *Thunnus thynnus thynnus* and *T. alalunga*. *Marine Biology* **143,** 613–621.
Rice, W. R. (1989). Analyzing tables of statistical test. *Evolution* **43,** 223–225.
Schneider, S., Roessli, D. & Excoffier, L. (2000). *Arlequin Version 2.000: A Software for Population Genetics Data Analysis*. Geneva: Genetics and Biometry Laboratory, University of Geneva.
SCRS (1997). Report of 1996 SCRS albacore stock assessment session. *ICCAT Collective Volume of Scientific Papers* **46,** 1–94.
Suzuki, Z., Warashina, Y. & Kishida, M. (1977). The comparison of catches by regular and deep tuna longline gears in the western and central equatorial Pacific. *Bulletin of Far Seas Fishery Research Laboratory* **15,** 51–89.
Takagi, M., Okamura, T., Chow, S. & Taniguchi, N. (2001). Preliminary study of albacore (*Thunnus alalunga*) stock differentiation inferred from microsatellite DNA analysis. *Fishery Bulletin US* **99,** 697–701.
Takeyama, H., Chow, S., Tsuzuki, H. & Matsunaga, T. (2001). Mitochondrial DNA sequence variation within and between *Thunnus* tuna species and its application to species identification. *Journal of Fish Biology* **58,** 1646–1657. doi: 10.1006/jfbi.2001.1575
Viñas, J., Santiago, J. & Pla, C. (1999). Genetic characterization and Atlantic-Mediterranean stock structure of albacore, *Thunnus alalunga*. *ICCAT Collective Volume of Scientific Papers* **49,** 188–191.
Viñas, J., Alvarado Bremer, J. R. & Pla, C. (2004). Inter-oceanic genetic differentiation among albacore (*Thunnus alalunga*) populations. *Marine Biology* **145,** 225–232.