Marine Biology (1997) 129: 221–232

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Mitochondrial DNA variation in king mackerel (*Scomberomorus cavalla*) from the western Atlantic Ocean and Gulf of Mexico

Received: 17 December 1996 / Accepted: 2 April 1997

Abstract King mackerel (*Scomberomorus cavalla* Cuvier) collected in 1992 and 1993 from 13 localities along the Atlantic coast of the southeastern USA and in the northern Gulf of Mexico were surveyed for variation in mitochondrial (mt)DNA and a nuclear-encoded dipeptidase locus (*PEPA*-2). Both polymorphic and fixed mtDNA restriction sites were identified and mapped using conventional and polymerase chain-reaction (PCR)-based methods. Heterogeneity in mtDNA haplotype frequencies was found only in comparisons of pooled haplotypes from Atlantic localities versus pooled haplotypes from Gulf localities. This finding indicates weak genetic divergence between king mackerel from the Atlantic and those from the Gulf. Frequencies of two *PEPA*-2 alleles essentially paralleled previous findings: one allele (*PEPA*-2a) was common among samples from western Gulf localities, whereas the other allele (*PEPA*-2b) was common among samples from Atlantic and eastern Gulf localities. There was considerable variation in *PEPA*-2 allele frequencies within broadly-defined regions. Variation in mtDNA haplotypes and *PEPA*-2 genotypes was independent, as was variation in mtDNA haplotypes with sex or age of individuals. Variation in *PEPA*-2 genotypes was not independent of sex or age of individuals. The latter result suggests that frequencies of *PEPA*-2 alleles in samples of king mackerel may stem, in part, from sex and age distributions of individuals within samples, and indicates that caution should be exercised in using allelic variation at *PEPA*-2 as a measure of population (stock) structure in king mackerel. The discordance in spatial patterning of mtDNA haplotypes versus *PEPA*-2 alleles across the Gulf (i.e. homogeneity in mtDNA haplotype frequencies versus heterogeneity in *PEPA*-2 allele frequencies) may be due to either female excess at several localities, sex-biased migration, or both. Observed patterns of genetic variation also are consistent with the hypothesis that king mackerel in the western Atlantic may have been subdivided during Pleistocene glaciation, and that the current distribution of *PEPA*-2 alleles may be a historical artefact.

Communicated by N.H. Marcus, Tallahassee

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Introduction

The king mackerel (*Scomberomorus cavalla* Cuvier) is a widely distributed, coastal pelagic fish of considerable economic importance. The species occurs in the western Atlantic Ocean from the Gulf of Maine to Brazil, and throughout both the Gulf of Mexico and the Caribbean Sea (Rivas 1951; Collette and Nauen 1983). Both recreational and commercial catches of king mackerel in U.S. waters are substantial, and recreational landings are generally greater than commercial catches (Manooch 1979). Commercial catches in Mexico also are substantial (Collins and Trent 1990).

Present management of king mackerel fisheries in U.S. waters is based on a two migratory unit (stock) hypothesis: one stock (Atlantic Migratory Unit) occurs in the U.S. southeastern Atlantic (Atlantic); whereas the other (Gulf of Mexico Migratory Unit) occurs in the Gulf of Mexico (Gulf). The hypothesis of two migratory units is based primarily on mark-and-recapture studies (Williams and Godcharles 1984; Sutter et al. 1991; Schaefer and Fable 1994). Movement patterns of king mackerel in U.S. waters have been discussed extensively (Williams and Godcharles 1984; Fable et al. 1987, 1990; Sutter et al. 1991). Briefly, spring and summer movements probably reflect migration to spawning areas in the South Atlantic Bight (Collins and Stender 1987) and northern Gulf of Mexico (Grimes et al. 1990), followed by a fall return to wintering areas off southeastern and southern Florida and in the western Gulf off the Yucatan peninsula. Considerable movement also occurs across the northern Gulf, and overwintering groups have

been documented both along the U.S. Atlantic coast and in the northern Gulf (Grimes et al. 1990). No tags from king mackerel released in the southeastern U.S. waters have ever been returned from the Caribbean, suggesting little movement of king mackerel from the U.S. southeast Atlantic into the Caribbean Sea (Trent et al. 1983).

Genetic evidence in the form of electrophoretic patterns of a nuclear-encoded, polymorphic dipeptidase locus (*PEPA*-2) has been interpreted to suggest that there are two stocks of king mackerel in the Gulf, one in the eastern Gulf and the other in the western Gulf (Johnson et al. 1993). Two common alleles at *PEPA*-2 were found to vary in frequency within the U.S. king mackerel fishery: one (*PEPA*-2a) was in high frequency among king mackerel sampled from the western and northwestern Gulf; whereas the other allele (*PEPA*-2b) was in high frequency among king mackerel sampled from the Atlantic and the northeastern Gulf. Johnson et al. hypothesized the occurrence of a western Gulf stock with wintering grounds in Mexican waters that migrated northward during the spring and summer; and an eastern Gulf stock with wintering grounds in waters off southern Florida that migrated northward along both coasts of Florida during the spring. Studies of its early life history (Grimes et al. 1988, 1990) are consistent with the notion of two spawning groups of king mackerel in the Gulf, i.e. one in the north-central Gulf and one in Mexican waters.

The electrophoretic data of Johnson et al. (1993) did not distinguish king mackerel in the eastern Gulf from those in the Atlantic, suggesting that king mackerel in the Atlantic do not represent a distinct unit or stock. However, recent mark-and-recapture data (Schaefer and Fable 1994) support earlier experiments (Williams and Godcharles 1984; Sutter et al. 1991) that showed relatively little movement of king mackerel from the Gulf into the Atlantic and vice-versa. In addition, DeVries and Grimes (1997) have shown recently that growth rates in both sexes differ significantly among king mackerel sampled from the U.S. Atlantic, the eastern Gulf, and the western Gulf. Persistence of this pattern over a 15 yr period (from 1977 to 1992) suggested to the authors that three stocks of king mackerel may exist in U.S. waters (DeVries and Grimes 1997).

In this study, we assessed variation in mitochondrial (mt)DNA among king mackerel sampled from the southeastern U.S. Atlantic, the northern Gulf, and two localities in Mexican waters. The purpose of the study was to document the mtDNA-based genetic structure of the U.S. king mackerel fishery as a means of testing hypotheses of king mackerel population-structure. The use of mtDNA analysis in assessing marine fish population structure is well documented both theoretically and empirically (Avise 1987; Ovenden 1990; Smolenski et al. 1993; Gold et al. 1994; Graves and McDowell 1995).

Materials and methods

Samples of *Scomberomorus cavalla* Cuvier were procured from 13 localities in the southeastern U.S. Atlantic and Gulf of Mexico (Fig. 1; Table 1). Sampling occurred during 1992 except for one sample from Grand Isle, Louisiana (Grand Isle-2, Table 1) taken in 1993. The sample from the Florida Keys was obtained during late winter; the remainder were obtained during spring, summer, and early fall. For fish sampled in U.S. waters, tissues (heart and muscle) were removed, placed into individual cryopreservation tubes, frozen in liquid nitrogen, and transported to College Station, Texas, for storage at −80 °C. Tissues removed from samples taken in Mexican waters were stored in blast-freezers in local fish houses, and then transported to College Station on wet ice. Because the trip from Mexico to College Station required up to 6 h traveling time (resulting in thawing of frozen tissues), reliable electrophoretic patterns of the nuclear-encoded *PEPA*-2 locus could not be obtained from half of the muscle-tissue samples taken at Veracruz nor for > 70% of the muscle-tissue samples taken at Merida (Table 1).

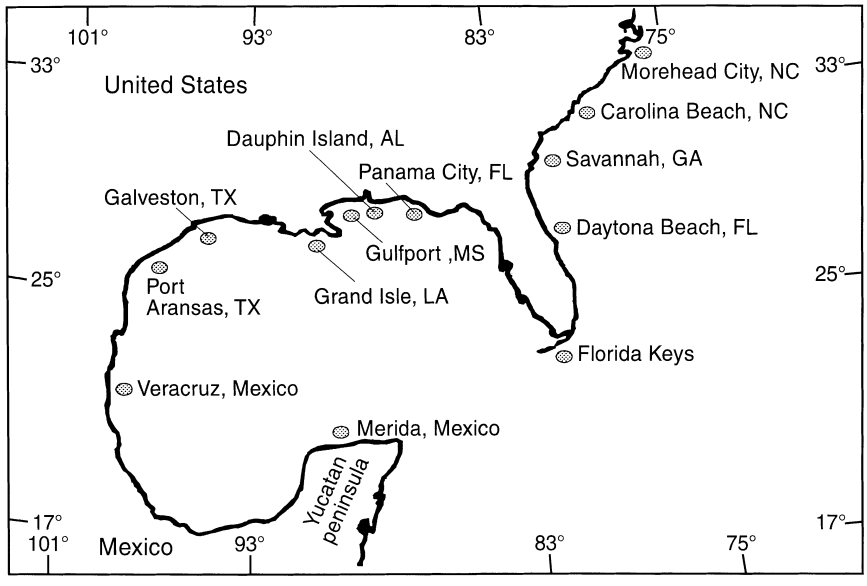

Fig. 1 Sampling localities for king mackerel, *Scomberomorus cavalla*, in present study (*NC* North Carolina; *GA* Georgia; *FL* Florida; *AL* Alabama; *MS* Mississippi; *LA* Louisiana; *TX* Texas)

Table 1 *Scomberomorus cavalla*. Sampling localities and number of individuals assayed for variation in mtDNA and *PEPA*-2 [*Charter* charter-boat day trips; *Tournament* 1 to 2 d competitions for large fish; *Commercial* commercial small-boat fishers; *state abbreviations* as in legend to Fig. 1]

Locality	Method of procurement	No. of individuals assayed	
		MtDNA	*PEPA*-2
S.E. Atlantic			
Morehead City, NC	Charter	50	47
Carolina Beach, NC	Tournament	55	50
Savannah, GA	Tournament	50	49
Daytona Beach, FL	Charter	47	42
Gulf of Mexico			
Florida Keys	Charter	50	45
Panama City, FL	Charter	55	46
Dauphin Island, AL	Charter	31	30
Gulfport, MS	Tournament	52	48
Grand Isle, LA (Sample 1)	Tournament	52	49
(Sample 2)	Tournament	31	27
Galveston, TX	Tournament	59	57
Port Aransas, TX	Tournament	51	50
Veracruz, Mexico	Commercial	52	26
Merida, Mexico	Commercial	43	12
Total		678	578

Approximate ages of individuals sampled from all localities except for the sample from Merida, Mexico and the 1993 sample from Grand Isle, Louisiana (Grand Isle-2) were determined by otolith-increment analysis. All otolith analyses were carried out by D. DeVries of the Panama City Laboratory of the Southeast Fisheries Science Center (National Marine Fisheries Service). Age/length data were taken from most individuals and may be obtained from the Panama City Laboratory. Sex was recorded for fish from three samples from the Atlantic (Morehead City, Carolina Beach, and Savannah), four samples from the western Gulf (Gulfport, Grand Isle-1, Grand Isle-2, and Veracruz), and the sample from the Florida Keys.

Mitochondrial (mt)DNA analysis

A total of 678 individuals were assayed for mtDNA fragment patterns following methods in Gold and Richardson (1991). Fourteen Type II restriction endonucleases (*Alw*44I, *Apa*I, *Bcl*I, *Dra*I, *Eco*RI, *Eco*RV, *Hpa*I, *Kpn*I, *Nco*I, *Pvu*II, *Sph*I, *Ssp*I, *Stu*I, and *Xba*I) were used individually to digest 1.0 to 1.5 μg of DNA in 40 μl reactions according to manufacturer's specifications. Fragment sizes for variant single-digestion patterns (designated by letter codes) of each restriction endonuclease may be obtained upon request from the first author. Methods of DNA digestion, agarose electrophoresis, Southern transfer to nylon filters, hybridization, and autoradiography are also in Gold and Richardson (1991). The probe used was an entire king mackerel mtDNA molecule cloned into lambda bacteriophage. *Hin*dIII-digested lambda DNA was used as a molecular weight marker.

Homology of fragment patterns from single digestions of mtDNA molecules was tested by multiple, side-by-side comparisons of variant patterns produced by each endonuclease enzyme. Homology was assumed if fragments of <14 kilobases were of the same size in side-by-side comparisons. Variant patterns exhibiting only a single band of >14 kilobases were tested for homology by double digestion with *Eco*RI. Restriction sites were mapped as described by Kristmundsdóttir et al. (1996).

Analysis of mtDNA data was facilitated by the "Restriction Enzyme Analysis Package" (REAP) of McElroy et al. (1992). Individual mtDNA genotypes (haplotypes) were identified by differences in restriction fragment (site) patterns. Homogeneity of haplotype distributions among (geographic) sampling localities was tested using a Monte Carlo randomization procedure (Roff and Bentzen 1989) and the log-likelihood (*G*)-test (Sokal and Rohlf 1981). Significance levels for multiple tests performed simultaneously were adjusted using the sequential Bonferroni approach (Rice 1989). F_{ST} values, a measure of the variance in mtDNA frequencies, were calculated using equations in Weir and Cockerham (1984) and computer programs described in Weir (1990).

Restriction-site presence/absence (binary) matrices for estimating nucleotide-sequence divergence and for phylogenetic and phenetic analyses were constructed using individual mtDNA haplotypes and individual sample localities as operational units. Maximum-parsimony analysis of both matrices employed Version 3.0s of the "Phylogenetic Analysis Using Parsimony" (PAUP) program of Swofford (1991). Nucleotide-sequence divergence among mtDNA haplotypes was estimated using restriction sites (Nei and Li 1979). Nucleotide-sequence divergence among samples (interpopulational divergence) was generated after Nei and Tajima (1981). Similarity analysis of matrices of nucleotide-sequence divergence among haplotypes and among samples employed the neighbor-joining algorithm (Saitou and Nei 1987) in Version 3.4 of the "Phylogenetic Inference Package" (PHYLIP) of Felsenstein (1992) and UPGMA (unweighted pair-group method using arithmetic averages) clustering (Sneath and Sokal 1973).

Spatial autocorrelation analysis of frequencies of common mtDNA haplotypes was used to determine whether haplotype frequencies at any sampling locality were independent of haplotype frequencies at neighboring sampling localities. The analysis was carried out using the "Spatial Autocorrelation Analysis Program" (SAAP) of Wartenberg (1989), and involved computation of autocorrelation (Moran's I) coefficients as a function of geographic distance between pairs of localities. To minimize "noise" generated by low-frequency haplotypes, autocorrelation coefficients were generated only from haplotypes found in ≥4 individuals (12 haplotypes among king mackerel from the Atlantic, and 14 haplotypes among king mackerel from the Gulf). Two SAAP runs were carried out: the first employed equal numbers of pairwise comparisons in each distance class; the second employed equal geographic distances between distance classes. Distance classes were generated by SAAP from input latitude and longitude of each sampling locality.

Allozyme analysis

Horizontal starch-gel electrophoresis was used to resolve allele products of the nuclear-encoded dipeptidase locus (*PEPA*-2) used by Johnson et al. (1993). Preparation of tissue (muscle) samples and horizontal electrophoresis followed procedures in Morizot and Schmidt (1992). Gels consisted of 72 g starch, 60 ml electrophoresis buffer [*N*-(3-aminopropyl)-morpholine-citrate, pH 6.1 (Clayton and Tretiak 1972)], and 540 ml distilled water (May 1990). Gels

were run at 20 to 25 mA and 270 to 320 V for 4 h at 4 °C. After electrophoresis, gels were sliced horizontally into three sections and stained for glycyl-leucine peptidase following Morizot and Schmidt (1992). Because allele products at *PEPA*-2 are not easily resolved, about half the individuals were assayed at least two separate times. Nomenclature for the two common alleles at *PEPA*-2 follows that of Johnson et al. (1993), viz. allele *a* is the more anodal.

Tests of Hardy–Weinberg equilibrium expectations, Wright's F_{ST} statistic, and Nei's (1978) unbiased genetic-distance values were generated using the BIOSYS-1 program of Swofford and Selander (1981). Homogeneity of genotype and allele distributions among (geographic) sampling localities was tested using the Monte Carlo randomization procedure (Roff and Bentzen 1989) and log-likelihood test (Sokal and Rohlf 1981). Significance levels for multiple tests performed simultaneously were adjusted after Rice (1989).

Tests of independence

Independence of several parameters was tested individually using the Monte Carlo randomization procedure (Roff and Bentzen 1989) and log-likelihood test (Sokal and Rohlf 1981). Parameters included mtDNA haplotypes, *PEPA*-2 genotypes, localities, sex (or sex ratio), and specimen age. For the latter, individuals were divided into four groups based on age in years: Group 1 (Ages 0 to 3), Group 2 (Ages 4 to 6), Group 3 (Ages 7 to 9), and Group 4 (Ages ≥10). Subdivision into age groups was necessary to increase cell sizes for more rigorous statistical testing. Individual tests of independence included: (i) mtDNA haplotypes versus *PEPA*-2 genotypes, (ii) mtDNA haplotypes or *PEPA*-2 genotypes versus locality, sex, or age, and (iii) locality or sex versus age.

Results

MtDNA analyses

MtDNA fragment patterns produced by single-digestion with all 14 restriction endonucleases were used to generate 122 mtDNA composite digestion patterns (haplotypes) for *Scomberomorus cavalla*. No evidence for size variation in the mtDNA molecule was observed among the individuals surveyed. Of the 122 different mtDNA haplotypes, 1 was found in >100 individuals, 7 were found in 25 to 75 individuals, 40 were found in 2 to 15 individuals, and 74 were found in only 1 individual each. A listing of all haplotypes, digestion patterns for each restriction enzyme, and the distribution of haplotypes across sample localities is available upon request from the senior author. The percentage nucleotide-sequence divergence (distance) among the 122 haplotypes ranged from 0.132 to 2.474, with a mean (±SE) of 0.722 (± 0.004).

Intrapopulational nucleotide-sequence diversities varied from 0.443 ± 0.249 (SD) in the sample from Savannah, Georgia to 0.555 ± 0.375 in the sample from Carolina Beach, North Carolina (Table 2). Intrapopulational diversities at all localities were within one standard deviation of each another, indicating that levels of mtDNA variation are essentially identical throughout the geographic area surveyed. Results of bootstrap analyses and log-likelihood tests for spatial homogeneity in mtDNA haplotype frequencies are shown in Table 3. Tests were carried out: (i) among all sampling localities; (ii) among sampling localities within defined regions, i.e. Atlantic (Morehead City, Carolina Beach, Savannah, and Daytona Beach), Gulf [Florida Keys, Panama City, Dauphin Island, Gulfport, Grand Isle (both samples pooled), Galveston, Port Aransas, Veracruz, and Merida], East Gulf (Florida Keys, Panama City, and Dauphin Island), and West Gulf [Gulfport, Grand Isle (both samples pooled), Galveston, Port Aransas, Veracruz, and Merida]; and (iii) between defined groups where sampling localities were pooled. Defined groups in the latter were the same as above, except that the sample from the Florida Keys was not included in the "Gulf" group. Pooling samples in this way served to: (i) increase the number of individuals within test groups, thus increasing rigor of homogeneity testing, and (ii) permit testing of regional groupings in relation to hypotheses of stock structure of king mackerel in U.S. waters.

Following corrections for multiple tests, significant heterogeneity was found only in log-likelihood tests of all localities and of pooled comparisons of (i) samples

Table 2 *Scomberomorus cavalla*. Number of mtDNA haplotypes (*N*), intrapopulational (mtDNA) nucleotide-sequence diversities, and frequencies of *PEPA*-2 alleles among samples of king mackerel from U.S. southeastern Atlantic and Gulf of Mexico

Locality	mtDNA		*PEPA*-2, allele frequency	
	(*N*)	Nucleotide sequence diversity[a]	*PEPA*-2a	*PEPA*-2b
Morehead City	(20)	0.45 ± 0.24	0.011	0.989
Carolina Beach	(29)	0.55 ± 0.37	0.360	0.640
Savannah	(19)	0.44 ± 0.25	0.337	0.663
Daytona Beach	(21)	0.46 ± 0.26	0.071	0.929
Florida Keys	(17)	0.47 ± 0.25	0.022	0.978
Panama City	(21)	0.48 ± 0.26	0.043	0.957
Dauphin Island	(17)	0.47 ± 0.26	0.133	0.867
Gulfport	(22)	0.47 ± 0.27	0.469	0.531
Grand Isle-1	(28)	0.49 ± 0.28	0.490	0.510
Grand Isle-2	(14)	0.47 ± 0.28	0.296	0.704
Galveston	(28)	0.51 ± 0.27	0.421	0.579
Port Aransas	(28)	0.49 ± 0.26	0.510	0.490
Veracruz	(23)	0.45 ± 0.25	0.558	0.442
Merida	(19)	0.51 ± 0.26	0.524	0.458

[a] Mean ± SD

Table 3 *Scomberomorus cavalla*. Tests for spatial homogeneity in mtDNA haplotype frequencies among king mackerel from U.S. southeastern Atlantic and Gulf of Mexico [(N) no. of samples; P probability based on 1000 bootstrap replicates; F_{ST} measure of variance in mtDNA haplotype frequencies]

Test group	(N)	P	Probability from G-tests	F_{ST}
All localities[a]	(13)	0.021[b]	≃0.005	−0.013
Atlantic localities	(4)	0.063	≃0.052	−0.015
Gulf localities	(9)	0.096	≃0.030[b]	−0.012
East Gulf localities	(3)	0.055	≃0.027[b]	−0.014
West Gulf localities	(6)	0.062	≃0.050[b]	−0.011
Pooled comparisons				
Atlantic vs East Gulf	(2)	0.054	≃0.004	−0.001
East Gulf vs West Gulf	(2)	0.564	≃0.020[b]	−0.001
Atlantic + Florida Keys vs Gulf[c]	(2)	0.263	≃0.127	−0.001
Atlantic + East Gulf vs West Gulf	(2)	0.084	≃0.081	−0.001
Atlantic vs Florida Keys + Gulf[c]	(2)	0.048[b]	≃0.007	0.000

[a] Samples from Louisiana pooled
[b] Non-significant ($P > 0.05$) when corrected for multiple tests
[c] Gulf does not include sample from Florida Keys

from the Atlantic versus samples from the eastern Gulf, and (ii) samples from the Atlantic versus the sample from the Florida Keys combined with samples from the remainder of the Gulf (Table 3). All tests of homogeneity among samples within defined regions were non-significant, as were other tests between defined regions where sampling localities were pooled. Included in the latter were comparisons between sampling localities from the eastern Gulf versus those from the western Gulf, and between sampling localities from the Atlantic and eastern Gulf versus those from the western Gulf. These two comparisons represent a test of the hypothesis of Johnson et al. (1993) that two stocks of king mackerel exist in the Gulf of Mexico. Estimates of F_{ST} were either negative or zero (Table 3), indicating significant gene flow among comparison groups. Results of homogeneity testing are consistent with the hypothesis that king mackerel from the U.S. southeastern Atlantic may be *weakly* subdivided from king mackerel in the Gulf. They are *not* consistent with the hypothesis that king mackerel in the Gulf are subdivided genetically.

Maximum-parsimony analysis of both haplotype and sample restriction-site (presence/absence) matrices yielded unresolved multichotomies, indicating virtually no phylogeographic structure. A neighbor-joining tree (Fig. 2A) and UPGMA phenogram (not shown) generated from the matrix of (mtDNA) genetic distances among samples also revealed no evidence of phylogeographic (or phenetic) structuring of geographic samples. With few exceptions, geographically-proximal sampling localities were not more closely related phylogenetically or phenetically to one another than to other, more geographically-distal sampling localities.

Fifty-six Moran's I values (14 mtDNA haplotypes × 4 distance classes) were generated in each of two SAAP runs using sample localities from the Gulf (including the Florida Keys) and mtDNA haplotypes found in four or more individuals. Mean Moran's I

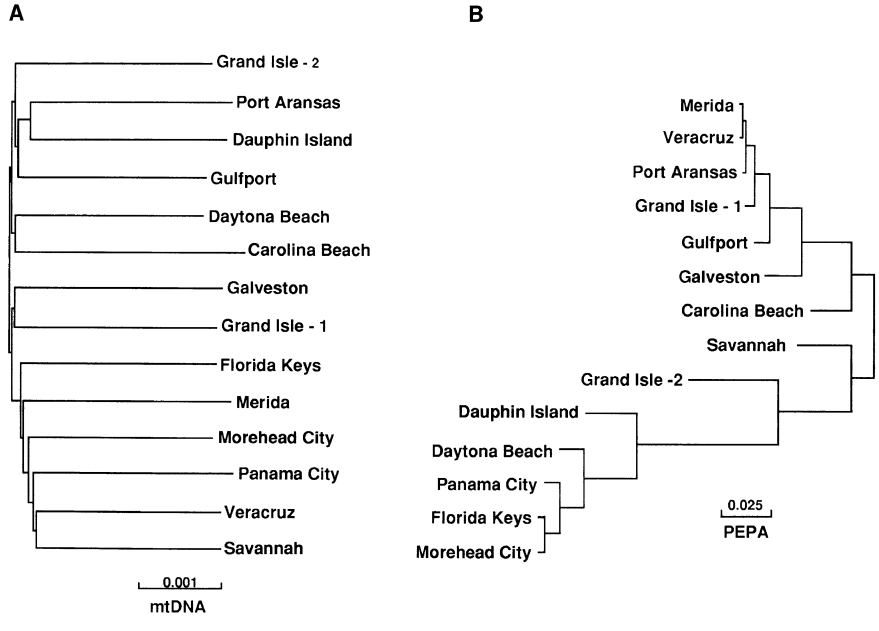

Fig. 2 *Scomberomorus cavalla*. Neighbor-joining trees generated from matrices of interpopulational (mtDNA) nucleotide-sequence divergence (**A**), and Nei's unbiased genetic (allozyme) distance (**B**). Bar scales below trees represent relative distance units for each tree

values for each distance class in both runs were not significant, indicating the effective absence of spatial autocorrelation (positive or negative) among mtDNA haplotypes in the Gulf. Virtually identical results were obtained from two SAAP runs using sampling localities from the Atlantic. Results from spatial autocorrelation analyses were consistent with the hypothesis of continuous gene flow among sampling localities within the Atlantic and within the Gulf.

Allozyme analyses

Frequencies of the two alleles at *PEPA*-2 observed at each of 14 geographic samples of king mackerel are given in Table 2. Following corrections for multiple tests, no significant deviations from expected Hardy–Weinberg distribution of genotypes at *PEPA*-2 were found at any sampling locality; significant deviations from Hardy–Weinberg expectations, however, were found in all pooled groupings (Table 4). In each case, *D* values were negative, indicating a deficiency of observed heterozygous genotypes relative to those expected under equilibrium conditions.

Following corrections for multiple tests, bootstrap analyses and/or log-likelihood tests revealed significant heterogeneity in *PEPA*-2 genotype distributions in all comparisons except for bootstrap analysis of pooled East Gulf localities; F_{ST} values for all localities and for localities from the Atlantic and Gulf were comparatively high (Table 5). Tests of allele-frequency homogeneity among samples from the eastern and western Gulf yielded probability values of < 0.05 without corrections for multiple tests (Table 5). The significant deviation from Hardy–Weinberg expectations among pooled groupings along with heterogeneity in allele frequencies and observed F_{ST} values indicate significant discontinuities in *PEPA*-2 allele distributions among king mackerel localities.

A neighbor-joining tree (Fig. 2B) generated from a matrix of Nei's unbiased genetic distance among samples revealed evidence of phylogeographic and phenetic structuring: sampling localities from the western Gulf (viz. Merida, Veracruz, Port Aransas, Grand Isle-1, Gulfport, and Galveston) are included within a cluster, as are sampling localities from the eastern Gulf and the Atlantic (viz. Savannah, Dauphin Island, Daytona Beach, Panama City, Florida Keys, and Morehead City). Only the sample from Carolina Beach (included in the western Gulf group) and the Grand Isle-2 sample (included in the Atlantic group) appear out-of-place geographically. Five of the sampling localities from the Atlantic and the eastern Gulf (viz. Morehead City, Florida Keys, Daytona Beach, Panama City, and Dauphin Island) and five of the sample localities from the western Gulf (viz. Gulfport, Grand Isle-1, Port Aransas, Veracruz, and Merida) formed cohesive clusters in an UPGMA phenogram (not shown).

Spatial variation in *PEPA*-2 allele frequencies observed in this study parallels that found by Johnson et al. (1993) in their study of *PEPA*-2 variation among nearly 9000 king mackerel sampled between 1985 and 1990 over approximately the same geographic range (Fig. 3 of present study). In general, king mackerel sampled from Atlantic and eastern Gulf localities appear to be characterized by low frequencies of Allele *PEPA*-2a, whereas king mackerel sampled from the western Gulf appear to possess higher frequencies of this allele. However, there appears to be considerable temporal fluctuation of *PEPA*-2 allele frequencies at a number of localities, and notably the range of *PEPA*-2a allele frequencies appears to be much greater among samples from the Gulf than among samples from the Atlantic. In addition, frequencies of *PEPA*-2a observed in this study at both Carolina Beach and Savannah were higher (Fig. 3) than those of any of the samples from the Atlantic examined by Johnson et al., whereas frequencies of *PEPA*-2a observed in this study among samples from the western Gulf were generally lower than those examined by Johnson et al.

Comparison of variation in mtDNA and *PEPA*-2

There were weak differences in mtDNA haplotype frequencies between king mackerel in the Atlantic

Table 4 *Scomberomorus cavalla*. Results of significance tests (exact probabilities) for Hardy–Weinberg equilibrium expectations and heterozygote deficiency (*D*) values at *PEPA*-2 among king mackerel from U.S. southeastern Atlantic and Gulf of Mexico [*Non-significant ($P > 0.05$) when corrected for multiple tests; *FK* Florida Keys; *GI-1* Grand Isle (1992 sample); *VC* Veracruz]

Test group	No. of samples	No. of tests	*P*	*D*
Individual samples	14	14	0.011 (FK)*	–
			0.021 (GI-1)*	–
			0.022 (VC)*	–
Pooled samples				
All localities	1	1	0.000	−0.326
Atlantic localities	1	1	0.000	−0.309
Gulf localities	1	1	0.000	−0.314
East Gulf localities	1	1	0.003	−0.396
West Gulf localities	1	1	0.007	−0.165

Table 5 *Scomberomorus cavalla*. Tests for spatial homogeneity in *PEPA*-2 genotype distributions among king mackerel from U.S. southeastern Atlantic and Gulf of Mexico [*P* probability based on 1000 bootstrap replicates; *non-significant ($P > 0.05$) when corrected for multiple tests]

Test group	*P*	Probability from *G*-test	F_{ST}
All localities	0.000	< 0.001	0.189
Atlantic localities	0.000	< 0.001	0.154
Gulf localities	0.000	< 0.001	0.174
East Gulf localities	0.041*	$\simeq 0.018$	0.037
West Gulf localities	0.014	$\simeq 0.021$	0.027

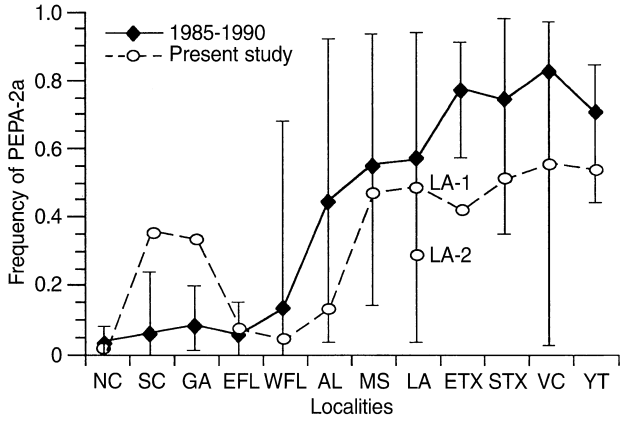

Fig. 3 *Scomberomorus cavalla*. Geographic variation at *PEPA*-2 [♦ frequencies of Allele *PEPA*-2a averaged over all individuals sampled at different localities between 1985 and 1990; *range bars* limits of frequencies of *PEPA*-2a in single samples of ≥10 individuals at each locality, data from Johnson et al. (1993); ○ frequencies of *PEPA*-2a observed at different localities in present study; *abscissa* approximate sampling localities by state acronym (see Fig. 1) except for *SC* (South Carolina), *EFL* and *WFL* (east and west Florida), *ETX* and *STX* (east Texas and south Texas), *VC* (Veracruz, Mexico), and *YT* (Yucatan Peninsula, Mexico); *ordinate* frequencies of *PEPA*-2a]

versus those in the Gulf, with no evidence of phylogenetic or phenetic cohesion of mtDNA haplotypes from geographically-proximate sampling localities. There were strong differences in *PEPA-2* allele frequencies within both Atlantic and Gulf localities, and phylogenetic or phenetic cohesion of samples from geographically-proximate localities. The lack of concordance between spatial variation in mtDNA and *PEPA*-2 is best exemplified by comparison of neighbor-joining trees (Fig. 2) where there is little correspondence between affinities or similarities of sampling localities based on mtDNA distances versus those based on *PEPA*-2 distances. In addition, the cophenetic correlation (r_{xy}) between the mtDNA and *PEPA*-2 distance matrices is -0.206 ± 0.104 (SE), and does not differ significantly from zero (Student's $t_{[.05,\ 89]} = 1.985$, $P > 0.05$).

Other analyses

Differences in pattern of spatial variation between mtDNA and an allozyme-encoding nuclear gene, where greater divergence among samples is observed in the nuclear gene, is unusual, as the reverse pattern (mtDNA divergence among conspecific samples exceeds that of allozyme divergence) is encountered more frequently (Saunders et al. 1986; Birky et al. 1989; Ward et al. 1989). To examine this in king mackerel further, variation in mtDNA haplotypes and *PEPA*-2 genotypes was tested for independence from each other, and from variation in sex (or sex ratio) and specimen age. Tests for independence of mtDNA haplotypes and *PEPA*-2 genotypes at individual localities were non-significant (data not shown), as were tests for independence when localities were pooled by region (Table 6).

Table 6 *Scomberomorus cavalla*. Tests of independence of mtDNA haplotypes and *PEPA*-2 genotypes among king mackerel from U.S. southeastern Atlantic and Gulf of Mexico [*P* probability based on 1000 bootstrap replicates; *non-significant ($P > 0.05$) when corrected for multiple tests]

Test group	*P*	Probability from *G*-tests
Atlantic localities	0.083	≃0.027*
Gulf localities	0.463	≃0.018*
East Gulf localities	0.157	>0.100
West Gulf localities	0.617	≃0.054

Tests for independence of mtDNA haplotypes and of *PEPA*-2 genotypes versus sex of individuals were limited to the eight localities for which sex data were available. These included three localities from the Atlantic (Morehead City, Carolina Beach, and Savannah), and five from the Gulf (Florida Keys, Gulfport, Grand Isle-1, Grand Isle-2, and Veracruz). Tests of independence (Table 7) revealed that variation in mtDNA haplotypes was independent of variation in sex. Variation in *PEPA*-2 genotypes was not: significant, non-random associations between *PEPA*-2 genotypes and sex were observed among all localities pooled and among localities from the Gulf (Table 7). *V*-tests (DeSalle et al. 1987) using arcsine, square-root-transformed *PEPA*-2 genotype frequencies indicated that among all localities there was a slight, but significant ($P \simeq 0.036$) association of *PEPA*-2b homozygotes with females, and a highly significant ($P \simeq 0.005$) association of *PEPA*-2a homozygotes with males. The same pattern was observed among localities from the Gulf, i.e. a weak ($P \simeq 0.047$) association between *PEPA*-2b homozygotes with females and a strong ($P \simeq 0.004$) association of *PEPA*-2a homozygotes with males. Examination of sex ratios (expressed as percentage females), and of *PEPA*-2 genotype distributions by sex, revealed that 6 of 8 samples had significant excesses of females (relative to an expected 1:1 sex ratio), and that *PEPA*-2 genotype distributions differed between sexes at several individual localities, among localities in the Gulf, and over all localities (Table 8).

Tests for independence of mtDNA haplotypes and of *PEPA*-2 genotypes versus age groups revealed that

Table 7 *Scomberomorus cavalla*. Tests for independence of mtDNA haplotypes and of *PEPA*-2 genotypes versus sex of king mackerel from U.S. southeastern Atlantic and Gulf of Mexico [*P* probability based on 1000 bootstrap replicates; *significant ($P < 0.05$) when corrected for multiple tests]

Test group	MtDNA		*PEPA*-2 genotypes	
	P	*G*-tests	*P*	*G*-tests
All localities	0.290	>0.100	0.013*	≃0.014*
Atlantic localities	0.510	>0.100	0.525	>0.100
Gulf localities	0.094	≃0.083	0.005*	≃0.012*

Table 8 *Scomberomorus cavalla*. Sex ratios and frequencies (by sex) of Allele *PEPA*-2b among king mackerel from U.S. southeastern Atlantic and Gulf of Mexico [*$P < 0.05$, based on chi-square goodness-of-fit tests to expected 1:1 sex ratio; **comparisons where distribution of *PEPA*-2 genotypes differed significantly ($P < 0.05$) between sexes, based on bootstrap and log-likelihood tests]

Sample	% females	*PEPA*-2b	
		♀♀	♂♂
Morehead City	68.1*	1.000	0.967
Carolina Beach	86.0*	0.674	0.429
Savannah	58.7	0.667	0.605
Florida Keys	80.0*	0.972	1.000
Gulfport	66.7*	0.650	0.333**
Grand Isle-1	73.9*	0.618	0.333**
Grand Isle-2	81.5*	0.727	0.600
Veracruz	52.0	0.346	0.583
All localities	71.6*	0.738	0.606**
Atlantic	71.3*	0.774	0.707
Gulf	71.8*	0.711	0.528**

Table 9 *Scomberomorus cavalla*. Tests for independence of mtDNA haplotypes and of *PEPA*-2 genotypes vs age groups of king mackerel from U.S. southeastern Atlantic and Gulf of Mexico [*P* probability based on 1000 bootstrap replicates; *non-significant ($P > 0.05$) when corrected for multiple tests]

Test group	MtDNA		*PEPA*-2 genotypes	
	P	*G*-tests	*P*	*G*-tests
All localities pooled	0.042*	>0.100	0.000	<0.001
Atlantic localities	0.899	>0.100	0.000	<0.001
Gulf localities	0.095	>0.100	0.000	<0.001

Table 10 *Scomberomorus cavalla*. Frequencies of *PEPA*-2b among age groups of king mackerel from U.S. southeastern Atlantic and Gulf of Mexico (*Values in parentheses* sample size; age groups with ≤5 individuals at a locality are not shown)

Sample locality	Ages (yr):			
	1–3	4–6	7–9	≥10
Morehead City	1.000 (16)	1.000 (16)	1.000 (8)	–
Carolina Beach	0.818 (11)	0.722 (9)	0.611 (9)	0.526 (19)
Savannah	0.719 (16)	0.800 (10)	0.550 (7)	0.389 (9)
Daytona Beach	0.900 (10)	0.929 (7)	–	–
Florida Keys	0.971 (34)	1.000 (6)	–	–
Panama City	0.959 (37)	–	–	–
Dauphin Island	0.929 (21)	–	–	–
Gulfport	0.800 (10)	0.650 (10)	0.333 (6)	0.382 (17)
Grand Isle-1	0.667 (15)	0.469 (16)	–	0.409 (11)
Galveston	0.667 (9)	0.643 (14)	0.437 (8)	0.412 (17)
Port Aransas	0.500 (17)	0.529 (17)	0.250 (8)	–
Veracruz	0.523 (18)	–	–	–

variation in mtDNA haplotypes was independent of variation in age, whereas variation in *PEPA*-2 genotypes among all localities and among both Atlantic and Gulf localities was not (Table 9). *V*-tests using arcsine, square-root-transformed *PEPA*-2 genotype frequencies demonstrated highly significant ($P < 0.001$) associations between *PEPA*-2b homozygotes and younger age groups, and between *PEPA*-2a homozygotes and older age groups in all three comparison groups, viz. among all localities and among localities from the Atlantic and the Gulf. This was borne out by examination of *PEPA*-2b allele frequencies in different age groups (Table 10), where younger fish tended to have higher frequencies of *PEPA*-2b at most localities. However, despite the age-group effect, it also is evident that within age groups, *PEPA*-2b allele frequencies vary clinally across the range of samples (Table 10).

Pairwise tests for independence among locality, sex ratio, and age groups revealed a significant ($P < 0.05$) non-random association between locality and sex ratio among Atlantic and Gulf localities, and a very highly significant ($P < 0.001$) non-random association between locality and age group in all comparisons (Table 11). The locality/sex-ratio effects probably reflect the high female percentage in the sample from Carolina Beach (Atlantic) and the high male percentage in the sample from Veracruz (Gulf) (Table 8). The locality/age group effect undoubtedly stems from the method of specimen procurement, as younger fish invariably were procured from charter-boat day trips and commercial fishers than were procured at tournaments (data not shown). Variation in sex ratios was independent of variation in age (Table 11).

Table 11 *Scomberomorus cavalla*. Tests for independence of locality, sex ratio, and age group of king mackerel from U.S. southeastern Atlantic and Gulf of Mexico [*P* probability based on 1000 bootstrap replicates; *non-significant ($P > 0.05$) when corrected for multiple tests]

Test group	Locality/sex ratio		Age group/sex ratio		Locality/age group	
	P	*G*-tests	*P*	*G*-tests	*P*	*G*-tests
All localities	0.040*	≃0.037*	0.717	>0.100	0.000	0.001
Atlantic localities	0.011	≃0.009	0.072	≃0.071	0.000	<0.001
Gulf localities	0.023	≃0.022	0.164	>0.100	0.000	<0.001

Discussion

Variation in mtDNA is consistent with the hypothesis (Williams and Godcharles 1984; Sutter et al. 1991; Schaefer and Fable 1994) that one stock of *Scomberomorus cavalla* occurs in the U.S. southeastern Atlantic, whereas another stock occurs in the Gulf of Mexico. A small difference in mtDNA haplotype distribution was detected in homogeneity tests between pooled samples from the Atlantic versus those from the Gulf (including both eastern and western Gulf localities). We emphasize that population subdivision between king mackerel in the Atlantic and Gulf is weak, in that: (i) homogeneity tests of mtDNA haplotype distributions carried out using individual samples were non-significant, (ii) F_{ST} values were either negative or zero in all comparisons, and (iii) there was no phylogeographic structure or disproportionate phenetic similarity of mtDNA haplotypes among king mackerel from geographically-proximate localities. Variation in mtDNA is not consistent with a second hypothesis (Johnson et al. 1993) that one stock of king mackerel occurs in the western Gulf, while another stock occurs in the eastern Gulf and southeastern Atlantic. MtDNA haplotype distributions across the Gulf are homogeneous, negative F_{ST} values occur in all comparisons, and no spatial autocorrelation exists among common haplotypes.

Genetic divergence between Atlantic and Gulf samples of several different marine species is either reported or known (Avise 1992; Gold et al. 1993 and unpublished data) and is undoubtedly due to historical or recent impediments to dispersal and gene flow. Possible impediments for any species include: (i) ocean-current patterns that are not conducive to movement between the southeastern Atlantic and Gulf, (ii) absence of suitable habitat somewhere between the two regions, or (iii) differences in biogeographic provinces separating Atlantic from Gulf fauna. Tagging data (Williams and Godcharles 1984; Sutter et al. 1991; Schaefer and Fable 1994) indicate relatively little movement of king mackerel between the Atlantic and Gulf, and to that extent are consistent with mtDNA data.

Geographic variation in the allozyme locus *PEPA*-2 was similar to that observed previously by Johnson et al. (1993): king mackerel from the Atlantic and eastern Gulf have lower frequencies of allele *PEPA*-2a than do king mackerel from the western Gulf, and a clinal pattern is generally evident. Differences between this and prior studies include a higher frequency of *PEPA*-2a alleles among fish from the southeastern Atlantic and a lower frequency of *PEPA*-2a alleles among fish from the western Gulf.

In this study, however, tests of independence of *PEPA*-2 genotypes with sex and age-group of specimens strongly indicated that observed frequencies of *PEPA*-2 genotypes among samples stem in part, from both the sex and age distribution of individuals within sample groups. Such non-independence of *PEPA*-2 genotypes with sex and age group could easily explain the temporal fluctuations in allele frequencies at the same localities observed by Johnson et al. (1993) and reinforced by results obtained in this study. Johnson et al., however, suggested that the temporal fluctuations might be due to sampling of different or mixed schools from different origins. Because eastern Gulf and western Gulf "stocks" (if they exist) would be expected to mix more frequently in the Gulf, this suggestion was consistent with their observation that the range of allele frequencies at the same localities over time was greater among Gulf localities than among Atlantic localities. Our findings of higher frequencies of *PEPA*-2a alleles at two localities in the Atlantic either argues against this suggestion, or indicates that western Gulf schools migrate as far north as Georgia and North Carolina.

Johnson et al. (1993) also found that *PEPA*-2 phenotypes generally were not correlated significantly with body length (suggesting no relation between *PEPA*-2 genotypes and age), nor did they find extensive evidence of non-random *PEPA*-2 allele distributions with sex. Our findings were the opposite, and at present, we have no testable hypotheses that account for the difference between the two studies. One possibility might be that age estimates based on total length are less reliable than estimates based on otolith-increment analysis. Johnson et al. also noted that individuals in some samples were small and difficult to sex.

The use of genetic markers to examine subpopulation (stock) structure rests largely on the assumption that variation at a genetic marker is affected only by population size or mutation rate. This assumption may be violated at *PEPA*-2, as specific *PEPA*-2 genotypes appear to be non-randomly associated with both sex and age of individuals – at least it was so in our study. The non-random association of *PEPA*-2 genotypes with sex is probably not due to sex linkage, as defined sex chromosomes are relatively rare in teleost fishes (Gold 1979). Some type of sex-related influence on allele expression, however, cannot be ruled out. Possible explanations for the observed non-random association with age are varied, and include: (i) selective differences among genotypes at different individual ages, and (ii) ontogenetic or developmental differences, where alternate alleles (in heterozygotes) are expressed at different ages. Both possibilities are consistent with the observation that the distribution of homozygous genotypes at *PEPA*-2 differs significantly with fish age. However, both possibilities would be difficult to test empirically in king mackerel, in part because of the generic (and unknown) biochemical function of the *PEPA*-2 locus, and in part because of the present impossibility of experimentally breeding and raising king mackerel under controlled conditions. Regardless, our finding that variation in *PEPA*-2 genotypes is constrained by sex and age would appear to limit the use of this locus in king mackerel stock-evaluation, and, in our view, considerable caution should be exercised in using allelic variation at the *PEPA*-2 locus as a measure of genetic stocks among king mackerel. If variation at

PEPA-2 continues to be used to assess genetic stocks of king mackerel, individuals should be subdivided into both sex and age groups.

Even though frequencies of *PEPA*-2 genotypes within samples in this study appear to be constrained by the sex and age distribution of individuals, frequencies of *PEPA*-2 alleles still appear to vary clinally across the range of samples, even when attempts are made to remove age-group effects. This geographic variation in allele frequencies at *PEPA*-2 is not concordant with geographic variation in mtDNA haplotypes. The lack of concordance between variation in mtDNA haplotypes and variation in *PEPA*-2 genotypes, and the observation that divergence among sample localities in *PEPA*-2 allele frequencies was greater than that in mtDNA haplotype frequencies, were unexpected findings of this study. In other studies that have employed both mitochondrial and allozyme markers to examine patterns of mtDNA and allozyme divergence among geographic samples of the same species, divergence (if it exists) typically occurs in the mtDNA and exceeds that of the allozymes. Notable examples include horseshoe crabs (Saunders et al. 1986), American oysters (Reeb and Avise 1990), and walleye (Ward et al. 1989). The usual explanation for the greater divergence in mtDNA is that the genetically-haploid state and matrilineal inheritance of mtDNA generates a smaller effective population size, making it more susceptible to genetic drift (Birky et al. 1989).

In king mackerel, divergence of the allozyme locus exceeds that of mtDNA. Studies where allozyme divergence within a species exceeds mtDNA divergence are rare, and largely include examples (Ovenden and White 1990; Ferguson et al. 1991) where allozyme divergence accompanied by near-uniformity of mtDNA haplotypes is explained by severe, but transitory, bottleneck events that differentially impacted levels of mtDNA variation. The only examples known to us where allozyme divergence exceeds that in mtDNA, but where sufficient mtDNA and allozyme variation exist to preclude a recent bottleneck, are yellowfin tuna (Ward et al. 1994) and minke whales (Bakke et al. 1996). In yellowfin tuna, Ward et al. documented significant divergence in a glucose-6-phosphate isomerase locus between samples from the eastern versus central/western Pacific. They found no difference in mtDNA haplotype frequencies, paralleling an earlier study of mtDNA divergence in yellowfin tuna by Scoles and Graves (1993), and suggested that the difference in allozyme versus mtDNA pattern could be due to different local selective processes. They opted for the possibility that further mtDNA analysis ultimately would reveal population substructuring. Bakke et al. found essentially the same pattern in minke whales from the North Atlantic, but simply noted the rarity of examples in which mtDNA exhibits significantly less substructuring than allozymes.

Under a neutral model (i.e. variation in genetically-based characters is affected only by effective population size and rates of mutation) and equilibrium conditions, allozyme divergence can exceed that of mtDNA if (i) the breeding sex ratio is strongly biased towards females, and/or (ii) there is a greater migration rate among females than among males (Birky et al. 1989). In king mackerel, the significant excess of females observed at most sample localities could account for difference in divergence between mtDNA and *PEPA*-2, provided there is either a similar sex-biased excess in the number of breeding adults, or the number of genetically-successful migrants, or both. Other studies (Johnson et al. 1983; Trent et al. 1983, 1987) also have reported significant excesses of females among samples of king mackerel, and Manooch et al. (1987) reported that mortality estimates were invariably lower for king mackerel females than for males. However, while females may outnumber males within the fishery, there are, at present, no data regarding the sex ratio of reproductively-successful adults. In addition, while there is ample evidence that females attain larger size more quickly than males (Trent et al. 1983; Manooch et al. 1987; DeVries and Grimes 1997), and hence might be capable of further or more extended migration, available mark-and-recapture data (Sutter et al. 1991) indicate that distance traveled by tagged king mackerel is not significantly related to size. Consequently, while one could hypothesize that the significant excess of females in the king mackerel fishery has contributed to the difference in pattern of divergence between mtDNA haplotypes and *PEPA*-2 alleles, there are no direct data that can be used to test the hypothesis.

One other possibility that merits note is that the allozyme divergence in king mackerel could reflect a historical pattern, whereas the mtDNA pattern reflects more contemporaneous events. In brief, king mackerel are warm-water, coastal pelagic fish that seldom are found in water < 20 °C (Manooch and Raver 1988). During the late Pleistocene, when waters on a reduced continental shelf in the northern Gulf (Rezak et al. 1985) would have been much cooler, king mackerel could have been isolated in warm-water refugia, perhaps off present-day southern Florida and the Yucatan peninsula. Given sufficient time, divergence would be expected in both mtDNA and nuclear-encoded genetic markers. Following glacial withdrawal, warmer waters and an expanding shelf in the northern Gulf would have provided suitable habitat for both feeding and spawning activities, resulting in the potential for gene flow between the putatively-isolated populations. Rates of approach to genetic homogeneity in frequency of both mtDNA and nuclear-encoded alleles would largely be a function of gene flow, and if there were large, female biases in breeding sex ratio, migration rate, or both, one might expect an accelerated rate of approach to homogeneity in frequencies of mtDNA alleles. Under this model, present-day conditions of king mackerel population (stock)-structure would be best reflected by mtDNA, and the *PEPA*-2 allele distribution best interpreted as an historical artefact. At least consistent with part of this model is that the two most divergent mtDNA haplo-

types uncovered in this study differed in (estimated) nucleotide sequence by 2.47%. Assuming that the rate of mtDNA-sequence evolution in homeothermic vertebrates [1% per lineage per million years (Wilson et al. 1985; Shields and Wilson 1987)] represents at least an upper bound of the mtDNA evolutionary rate in poikilothermic vertebrates (Martin and Palumbi 1993), the value of 2.47% would suggest a minimum age of king mackerel mtDNA lineages in the Atlantic and Gulf of roughly 1.2 million years, commensurate with Pleistocene glaciation.

Future genetic studies of king mackerel in U.S. waters should be designed to provide additional testing of spatial or temporal separation of Atlantic from Gulf samples. The next logical step would be to employ hypervariable, nuclear-encoded sequences such as microsatellite loci (Weber 1990; Wright and Bentzen 1994). These loci evolve more rapidly than either mtDNA or nuclear-encoded genes such as *PEPA*-2, such that variation at these loci could provide (i) more rigorous tests of contemporary population structure (of both sexes), and (ii) multiple markers for more accurately estimating proportions of distinct stocks over time in mixed fisheries (i.e. where boundaries of distinct stocks overlap). Future genetic studies should also examine the question of whether observed sex/age effects on variation at *PEPA*-2 are unique to that gene.

Acknowledgements We thank the following for direct and indirect assistance in obtaining specimens: K. Bailey, D. Bartee, K. Burns, M. Burton, L. Dukeheart, J. Franks, C. Furman, C. Grimes, A. Johnson, L. Mercer, M. Middlebrooks, L. Noble, T. Schmidt, E. Schroeder, R. Schuster, B. Shipp, M. Thiery, D. Thiessen, B. Thompson, S. Woodward, and B. Zales. Acknowledgments also are due to the following: North Carolina Division of Marine Fisheries, Coastal Resources Division of Georgia DNR, Islamorada Charter Boat Captains Association, Dauphin Island Marine Laboratory, Gulf Coast Research Laboratory, Louisiana State University, and the National Marine Fisheries Service. We also thank D. DeVries and C. Grimes for carrying out age determinations from otolith-increment analysis, and R. Broughton, E. Heist, and C. Grimes for comments on a draft of the manuscript. Work was supported primarily by the Marfin Program of the U.S. Department of Commerce (Grant NA17FF0385-1) and the Texas Agricultural Experiment Station (Project H-6703). The views expressed in the paper are those of the authors and do not necessarily reflect the views of the National Oceanic and Atmospheric Administration or any of its subagencies. The North Carolina Division of Marine Fisheries kindly provided funds for procurement of one sample from North Carolina. This paper is No. XVII in the series "Genetic Studies in Marine Fishes" and Contribution No. 54 of the Center for Biosystematics and Biodiversity at Texas A&M University.

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