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## Mitochondrial and nuclear DNA analysis of population subdivision among young-of-the-year Spanish mackerel (*Scomberomorus maculatus*) from the western Atlantic and Gulf of Mexico

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**Abstract** The degree of population subdivision among collections of young-of-the-year (YOY) Spanish mackerel *Scomberomorus maculatus* sampled from along the Atlantic coast of the United States (Atlantic) and Gulf of Mexico (Gulf) was examined. Young-of-the-year were collected from spawning areas to minimize possible mixing among putative spawning stocks that may occur at later life history stages along migratory pathways or at over-wintering grounds. Restriction fragment length polymorphism (RFLP) analysis of the mitochondrial DNA (mtDNA) ND4 region and a nuclear actin intron was conducted. Collections in each of two years were analyzed to minimize bias associated with sampling at a single point in space or time. Substantial variation was detected at ND4 (haplotype diversity = 0.81) and at the actin intron locus (gene diversity = 0.21). Significant differences in gene diversity or allele frequencies were not detected among temporal samples at any of three locations. A homogeneous distribution of genetic variance among samples from widely spaced geographic regions was consistent with the hypothesis that Spanish mackerel comprise a single intermingling genetic stock. Power analysis showed that the mitochondrial analysis from this study was much more likely to detect population subdivision than analysis of the nuclear actin locus, despite lower mtDNA sample size.

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### Introduction

The Spanish mackerel (*Scomberomorus maculatus*) is a migratory, coastal pelagic species that has supported major commercial and recreational fisheries in the United States since 1850 (Chittenden et al. 1993). Spanish mackerel occur seasonally along the east coast of the United States (Atlantic) from Cape Cod to Miami and in the Gulf of Mexico (Gulf) from Florida to the Yucatan Peninsula (Collette and Russo 1984). Knowledge of the underlying stock structure of this species is necessary to determine the appropriate units for conservation and management (Ovenden 1990).

Dispersal does not appear to be extensive during early life history stages of Spanish mackerel. Adults reach sexual maturity within 2–3 years (Powell 1975). Spawning occurs continuously for a period of several months during summer in waters shallower than 50 m (Baughman 1950; McEachran et al. 1980; Collins and Stender 1987). Eggs hatch within 25 h of release (Klima 1959), and larvae reach juvenile size (10 mm standard length) within 9 days (De Vries et al. 1990). Based on the presence of juveniles in estuarine nursery grounds, it is thought that larvae and juveniles migrate inshore (Hildebrand and Cable 1938; Wollam 1970).

During summer months, Spanish mackerel spawn in two or more geographically distinct regions that may represent discrete spawning stocks. In the Atlantic, spawning commences in April off Florida and the Carolinas, in mid-June in Chesapeake Bay, and in late August in the northernmost regions (Earll 1883; Powell 1975). Spawning continues in these regions until late summer. Extant biological information does not adequately address whether distinct spawning stocks exist along each region of the Atlantic coast or whether the entire coast represents a single stock (Lyles 1969; Chittenden et al. 1993). In the Gulf, spawning occurs in the coastal waters of Florida and Alabama (Wollam 1970; Dwinell and Futch 1973), Texas (McEachran et al. 1980), and Veracruz, Mexico (Mendoza 1968).

Spawning concentrations in the Atlantic and Gulf may represent genetically discrete spawning stocks (Wollam 1970).

Migratory patterns in Spanish mackerel may result in mixing of putative spawning stocks in the Atlantic and between the Atlantic and Gulf regions. The extent of coastal migration has not been well demonstrated due to a paucity of tagging information, but migration is thought to occur in large schools over great distances near the shore (Collette and Nauen 1983). Based on analysis of seasonal abundance, Spanish mackerel form winter concentrations off southern Florida (Beaumariage 1970) and Veracruz, Mexico (Mendoza 1968). These individuals presumably move northward with increasing springtime water temperatures along the Atlantic coast (Earll 1883; Beaumariage 1970), Gulf coast of Florida (Moe 1972) and western Gulf coast (Mendoza 1968). Southward return migrations occur in fall, as inferred by an individual tagged off Texas and recovered off Mexico (Sutherland and Fable 1980). In the Atlantic, the same concentration of fish may spawn continuously as they migrate up the coast in spring and summer. Alternatively, local spawning populations may remain distinct due to lack of migration from natal areas or because of natal homing tendencies (Chittenden et al. 1993). The extent to which individuals from geographically distinct spawning groups from the Gulf and Atlantic coasts mix off southern Florida during winter months is not well known.

Previous studies of the stock structure of Spanish mackerel have yielded conflicting results. Slight morphological differences were noted between collections of Spanish mackerel from the Atlantic and Gulf, using a total of 60 individuals (Collette and Russo 1984). Skow and Chittenden (1981) analyzed 70 fish from North Carolina and Mexico for a "fast and slow" hemoglobin phenotypic polymorphism and reported a significant difference in the distribution of alleles between samples. Nakamura (1987) undertook a much more detailed electrophoretic analysis, surveying nine polymorphic allozyme loci in 2,000 adult fish from six sample locations distributed along the Atlantic and Gulf coasts. In contrast to previous studies, significant differences in allele frequencies were not detected between Atlantic and Gulf samples. Conflicting results from these studies may be the result of different responses of the genetic and morphological characters to different environ-

mental influences along the range of the species (phenotypic plasticity; Barlow 1961; Clementi et al. 1994). Differences in sample design, sample sizes, and unknown sample bias may also have influenced the results.

The purpose of the present study was to elucidate the population structure of Spanish mackerel by evaluating genetic variation in large numbers of individuals collected with a sampling regime that was designed to minimize the confounding effects of adult migrations. A mitochondrial gene with a moderate level of polymorphism was desired for high-resolution genetic analysis. The four-fold lower effective population size of mitochondrial DNA (mtDNA) relative to nuclear DNA enhances the effect of genetic drift among isolated populations, thereby increasing the sensitivity of a population structure analysis. Because mtDNA inheritance is strictly maternal, however, we chose to analyze a nuclear actin intron locus as well, to examine gene flow within both sexes. Because stocks are likely to be maximally separated at the time of spawning, we chose to survey genetic variation within samples of young-of-the-year (YOY) Spanish mackerel collected at sites along the Atlantic and Gulf coasts. We assumed that at the time of collection, YOY Spanish mackerel have not undergone substantial mixing with those spawned in other areas. To determine both the degree to which a genetic signal varies among year classes at a location and among geographic locations, we chose to collect and analyze YOY from up to three successive year classes. The following null hypotheses were tested: (1) YOY Spanish mackerel taken from a single location in different years are genetically homogeneous; (2) Spanish mackerel sampled from different locations along the Atlantic coast comprise a single, genetically homogeneous stock; (3) Spanish mackerel sampled from Atlantic and Gulf coasts comprise a single, genetically homogeneous stock.

## Materials and methods

### Sampling

Spanish mackerel samples were collected over a period of 3 years from shrimp trawl bycatch and pound net fisheries (Table 1). Juveniles are reported to reach a maximum fork length of 30 cm by the end of the first summer's growth (Powell 1975; Fable et al.

**Table 1** *Scomberomorus maculatus* YOY collection information. (*n* = number of individuals)

Location	Year	Abbreviation	<i>n</i>	Average fork length in cm (range)	Collection method
Chesapeake Bay	1995	CB95	17	15.7 (3.8–26.0)	Trawl
	1996	CB96	48	25.1 (21.0–30.0)	Pound net
	1997	CB97	53	23.7 (15.5–27.7)	Pound net
North Carolina	1996	NC96	41	14.6 (12.0–18.0)	Trawl
South Carolina	1996	SC96	46	17.8 (13.0–24.5)	Trawl
	1997	SC97	54	18.6 (14.5–24.0)	Trawl
Georgia	1997	GA97	50	16.1 (13.0–25.6)	Trawl
Gulf Mexico	1996	GF96	17	25.8 (25.0–27.0)	Trawl
	1997	GF97	65	18.5 (12.5–24.5)	Trawl

1987). For this study, YOY, identified as those individuals with fork lengths less than 30 cm, were collected in the late summer and fall and presumably represented individuals spawned in late spring and early summer. Whole specimens were frozen at sea, transported to the laboratory on dry ice, and stored at  $-80^{\circ}\text{C}$  until analysis. White muscle tissue samples were placed in a storage buffer at room temperature for permanent storage (saturated NaCl, 250 mM EDTA, 20% DMSO). A total of 391 individuals were analyzed from southern Chesapeake Bay and the coasts of North Carolina, South Carolina, Georgia, and Texas. All individuals were less than 30 cm, and the average size was less than 20 cm for 8 of 11 collections (Table 1).

#### DNA isolation and amplification

Total genomic DNA for each sample was isolated from white muscle tissue. Tissues were incubated overnight in isolation buffer (Seutin et al. 1991) and DNA purified using phenol/chloroform extraction techniques. The polymerase chain reaction (PCR) was used to amplify target mitochondrial and nuclear gene fragments from genomic DNA. The PCR was performed using the BRL PCR Reagent System (Gibco BRL) with a final concentration of 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM  $\text{MgCl}_2$ , 0.2 mM dNTP mix, 1  $\mu\text{M}$  each primer, approximately 50 ng template DNA, and 1.125 units *Taq* polymerase in 50  $\mu\text{l}$  total volume. For ND4 amplification, cycling conditions included an initial denaturation of 5 min at  $95^{\circ}\text{C}$ , followed by 35 cycles of 1 min at  $95^{\circ}\text{C}$ , 1 min at  $51^{\circ}\text{C}$ , and 3 min at  $65^{\circ}\text{C}$ . Final extension was carried out for 7 min at  $72^{\circ}\text{C}$ . For actin amplification, annealing temperature was adjusted to  $65^{\circ}\text{C}$ .

#### Marker development

Introns of conserved actin genes have demonstrated substantial intraspecific polymorphism and have proven useful in population structure analysis (Palumbi and Baker 1994). Primers for an actin

intron originally developed for bluefin tuna (*Thunnus thynnus*; K. Reece, Virginia Institute of Marine Science) yielded several amplification products in Spanish mackerel. PCR products were cloned using TA cloning kits (Invitrogen) and positive clones were sequenced using Sequenase version 2.0 kits (U.S. Biochemical). The Spanish mackerel sequence was deposited in GenBank (accession number AF284445). The forward primer was redesigned based on a consensus sequence obtained for Spanish mackerel, sailfish (*Istiophorus platypterus*), and striped marlin (*Tetrapturus audax*) clones of the actin intron, using the program PC/GENE (Bairoch 1989), which resulted in a single degeneracy. No change was made to the reverse primer because it was set in the conserved actin exon region, whereas the forward primer overlapped an exon and variable intron region (Fig. 1). Primers for actin intron were forward 5'-CGGWCGCCCCGTCACCAGGTA-3' and reverse 5'-CCAGAGGCATACAGGGACAGCACAGC-3'. A single 617 base-pair (bp) product was amplified using the redesigned primers, of which the intron region accounted for 283 base pairs. The entire mitochondrial NADH dehydrogenase subunit 4 (ND4) and adjacent ND4L loci were amplified using the L 10421 primer from Bielawski and Gold (1996) and the primer 5'-CAAGAGTTTCAGGCTCC-TAAGACCA-3' (Bielawski, personal communication). For simplicity, we use the term "ND4" to refer to amplification of both ND4 and ND4L regions. A single product of approximately 1,500 bp was amplified.

#### Population screening

Variation at the ND4 and actin intron loci was assessed through restriction fragment length polymorphism (RFLP) analysis. Base substitutions at particular regions of DNA can result in gain or loss of restriction sites, generating different fragment patterns (RFLPs) for the amplified product upon digestion. Restriction digestions were performed following the manufacturer's protocols (Gibco BRL). Digestion products were separated electrophoretically on 2.5% NuSieve agarose gels (FMC BioProducts), stained with ethidium bromide, and visualized under ultraviolet light.

**Fig. 1** *Scomberomorus maculatus*. DNA sequence for actin intron. Original forward primer binding region, forward and reverse primer binding regions used in large scale survey, intron/exon junctions, *Hha*I restriction site, and partial amino acid sequence are shown above the sequence

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--Original Forward----->
                                -----Forward Primer----->
      A V F P S I V G R P R H Q|-----Intron----->
1  GCTGTCTTCC CGTCCATCGT CGGTCGCCCC CGTCACCAGG TAAATCAAGC TATTCATAAA

61  CTCCACGTGC CTA CTATCAT GAGCGAGTAT CGCTTTCAGT TGTTTACATC ACATTGCACA

121 ATCTATGGCA TAGGGTGAGA GCAAGATCCA CCGTGCGTAA TTGCGCACCA GAATTACGCA
                                |Hha I

181 TCTGAGCTTC TTATTACATG GAACTATTAC ACAGAAGACC AAATTCGTGT TTTTCCCATC

241 ATGGTAGTTA TGATGCTCTG ATGATAATCA ACTGTAGTTT TCCCACCACG CCCACTGATT
                                |-----Actin Exon----->
                                G V M V G M
301 CCTGGTCCTT GTATGTTATC AGGGCGTCAT GGTGGCATG GGCAGAAGGA CTCCTACGTC

361 GGACGAACGA GGCCAGAGA AAAAGAGGCA TCCTGACCCT GAAGTATCCC ATCGAGCAGC

421 GCATCATCAC CAACTGGGAC GACATGGAGA AGATCTGGCA CCACACCTTC TACAACGAGC

481 TGCGTGTGGC CCCCAGAGAG CACCCACCC TGGTGACAGA GGCCCTCTC AACCCAAAAG

541 CCAACAGAGA GAAGATGACC CAGATCATGT TCGAGACGTT CAATGTACCC GCAATGTACG
                                <-----Reverse Primer-----<
601 TCGCCATCCA GGCTGTGCTG TCCTGTATG CCTCTGG
  
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In a preliminary analysis, ND4 PCR products of eight Spanish mackerel from different collections were surveyed with a battery of 50 restriction endonucleases to identify those enzymes that had recognition sites within the amplified product. For the actin intron, a series of enzymes that recognized restriction sites within the sequenced clone were selected for analysis. Fifty individuals from the different collections were then screened with those enzymes with recognition sites in the ND4 or actin intron regions to identify those enzymes that produced RFLPs. Amplification products of all individuals were digested with those enzymes producing RFLPs in which the most common allele occurred at a frequency less than 0.95. Each individual was scored for the presence or absence of restriction sites to produce a composite haplotype for the mitochondrial ND4 locus and genotype for the actin intron locus. Because only polymorphic enzymes were chosen for analysis, diversity levels from this study may overestimate true diversity of the mtDNA and nuclear genomes.

#### Statistical analyses

Within-sample variation was estimated using haplotype diversity ( $h$ ) for mtDNA and expected heterozygosity ( $H_E$ ), or gene diversity, for nuclear DNA (Nei 1987). Both represent the probability that two alleles are different when randomly drawn from a sample. Standard errors of  $h$  and  $H_E$  were calculated according to Nei (1987; Eq. 8.12). To determine whether observed mtDNA  $h$  (or nuclear  $H_E$ ) values fell within expected 95% confidence intervals (CIs) over all samples, a resampling procedure was performed in the Resampling Stats environment, version 4.1b4 (Simon 1997). For each sample size, the original data was pooled, 1,000 permuted resamples were drawn of indicated size, and for each resample,  $h$  was calculated. Ninety-five percent CIs for  $h$  were then calculated from the 1,000 values. Ninety-five percent bootstrap CIs on allele frequencies were also generated (1,000 replicates with replacement).

For mtDNA, mean nucleotide sequence diversity was calculated ( $\pi$ ; Nei 1987), representing the average sequence divergence between haplotypes randomly drawn from a sample. The nucleotide sequence divergence between mtDNA haplotypes was calculated following Nei and Miller (1990) using the computer program REAP, version 4.0 (McElroy et al. 1991).

Proportion of genetic variance attributable to division among samples was calculated using variance in allele frequency ( $F_{ST}$ ; Weir and Cockerham 1984) and the number of restriction site differences for RFLP haplotype ( $\Phi_{ST}$ ; Excoffier et al. 1992). Estimates were obtained using the computer program Arlequin, version 1.1 (Schneider et al. 1997).

Exact-significance testing methods were used to evaluate conformance of genotypic proportions to Hardy-Weinberg equilibrium expectations and to evaluate homogeneity of temporal and spatial distributions of genetic variance. Unbiased estimators of exact significance probabilities for tests of Hardy-Weinberg equilibrium were used to evaluate the null hypothesis that alleles were randomly distributed among individuals at each locus. When considering a single, randomly mating population, this test may serve as a quality control to assure that alleles are codominant (Hare et al. 1996). Unbiased estimators of exact significance probabilities for tests of population homogeneity (Raymond and Rousset 1995a) were based on allele frequency only and did not incorporate allelic relatedness. These estimates were generated using the Markov chain algorithm described in Guo and Thompson (1993), as implemented in GENEPOP (Raymond and Rousset 1995b). To test for homogeneity in distribution of mtDNA restriction site variance among samples, the magnitude of the observed  $\Phi_{ST}$  statistic was compared to a null distribution of 10,000 values generated by permuting alleles among samples (Schneider et al. 1997). Probabilities across loci were combined using Fisher's method (Sokal and Rohlf 1995).

Significance testing took place at several hierarchical levels. Significance of inter-annual variation in allele frequencies was evaluated over all temporal samples at a location. After determining that temporal samples were not significantly different, these

samples were pooled. Significance of geographic variation was then determined among all Atlantic locations, for each pairwise comparison, and between Atlantic and Gulf samples pooled.

#### Power analysis

To investigate the sensitivity of the empirical analysis to migration, a computer simulation was performed. We used a model that simulated two populations under migration, mutation (infinite allele model), and genetic drift equilibrium (Buonaccorsi, unpublished data). Data sets from 100 simulated population pairs were generated for a range of migration rates, maintaining approximately the same diversity level ( $h$  or  $H$ ) as the mtDNA and nuclear empirical analyses of this study. A Roff and Bentzen (1989) chi-square analysis was performed on each population pair to determine significance of allele frequency heterogeneity ( $\alpha = 0.05$ ), taking the same sample sizes as for the Atlantic versus Gulf empirical analysis. Only polymorphic data sets (0.95 criteria) were considered.

## Results

### ND4 locus

Analysis of the mitochondrial ND4 locus with a panel of 50 restriction endonucleases revealed four enzymes that recognized polymorphic restriction sites at which the major allele occurred at a frequency less than 0.95: *HinfI*, *DdeI*, *StyI*, and *PstI*. The ND4 locus was successfully amplified and digested with all four enzymes for 376 individuals, resulting in 18 complete haplotypes (Table 2). Five haplotypes occurred at a frequency greater than 8% in the pooled sample, each different from the most common type by a single restriction site. Seven haplotypes were represented by a single individual.

RFLP analysis of the ND4 locus revealed considerable within-sample variation. Haplotype diversities and mean nucleotide sequence diversities of unpooled samples ranged from  $h = 0.760$  to  $0.882$  and  $\pi = 0.016$  to  $0.028$ , respectively (Table 3). Pooling temporal samples reduced the range of haplotype diversities to  $h = 0.775$ – $0.836$  (mean  $0.809$ ) and that of mean nucleotide sequence diversities to  $\pi = 0.023$ – $0.026$  (mean  $0.024$ ). All samples fell within expected 95% confidence intervals for the  $h$  statistic, indicating that the level of variation did not differ significantly among samples (Fig. 2).

To evaluate whether significant temporal divergence in allele frequencies existed between year classes, the distributions of haplotypes of YOY Spanish mackerel collected in different years at the same geographic location were compared. These included comparison of three year classes of YOY Spanish mackerel in Chesapeake Bay and two year classes in both South Carolina and the Gulf of Mexico (Table 2). No significant differences were found in the distribution of haplotypes among year-class samples at any location (Chesapeake Bay  $P = 0.88$ ; South Carolina  $P = 0.44$ ; Gulf of Mexico  $P = 0.12$ ). Temporal samples for each geographic location were pooled to increase the power of geographic analyses.

**Table 2** *Scomberomorus maculatus* ND4 composite haplotypes. Abbreviations follow Table 1.  $\Delta$  indicates number of restriction site changes from the common type (ABBA). Composite haplotypes reflect digestion with the following restriction endonucleases (left to

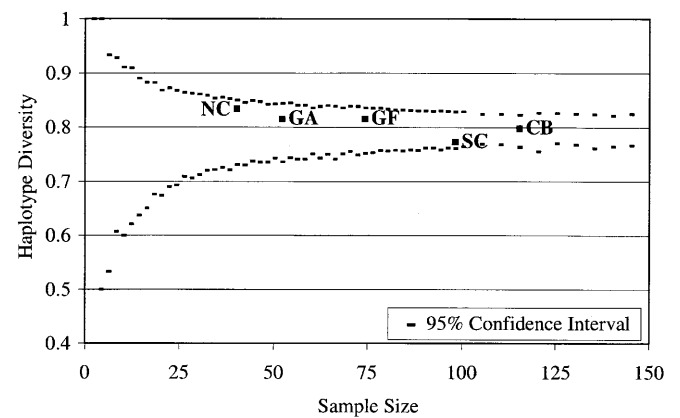
right): *Hinf*I, *Dde*I, *Sty*I, and *Pvu*II. Description of fragment sizes available from authors upon request. Parentheses indicate sum over years

	$\Delta$	CB95	CB96	CB97	(CB)	NC96	SC96	SC97	(SC)	GA97	GF96	GF97	(GF)	Total
ABBA	0	6	15	19	40	11	11	22	33	17	4	17	21	122
AABA	1	3	9	7	19	5	11	9	20	9	4	14	18	71
ABAA	1	0	9	8	17	9	13	10	23	6	2	12	14	69
BBBA	1	4	7	9	20	7	5	8	13	7	1	9	10	57
ABBB	1	3	3	5	11	4	3	3	6	4	3	2	5	30
ABBE	1	0	2	1	3	1	0	0	0	2	0	0	0	6
ABBC	2	0	1	2	3	0	0	0	0	0	1	0	1	4
BBAA	2	0	1	1	2	1	0	0	0	0	0	0	0	3
CABA	2	0	0	1	1	0	0	1	1	0	0	1	1	3
ABBF	1	0	0	0	0	1	0	0	0	0	0	1	1	2
BBBE	2	0	0	0	0	0	0	0	0	1	0	1	1	2
AAAA	2	0	0	0	0	0	0	0	0	0	1	0	1	1
ABBD	1	0	0	0	0	0	0	0	0	1	0	0	0	1
ACBA	1	0	0	0	0	0	0	1	1	0	0	0	0	1
ACBG	2	0	0	0	0	0	0	0	0	0	0	1	1	1
ADBA	1	0	0	0	0	0	0	0	0	1	0	0	0	1
BABA	2	0	0	0	0	1	0	0	0	0	0	0	0	1
BBAE	3	0	0	0	0	0	0	0	0	1	0	0	0	1
Total		16	47	53	116	40	43	54	97	49	16	58	74	376

**Table 3** *Scomberomorus maculatus* YOY sample size ( $n$ ) and within-sample diversity values for the ND4 locus. Sample abbreviations follow Table 1

Location	$n$	Haplotype diversity ( $h$ )	Standard error	Mean nucleotide sequence diversity ( $\pi$ )
CB95	16	0.775	0.0569	0.016
CB96	47	0.813	0.0293	0.024
CB97	53	0.806	0.0340	0.024
CB	116	0.800	0.0204	0.023
NC96	40	0.836	0.0285	0.026
SC96	43	0.777	0.0258	0.025
SC97	54	0.760	0.0376	0.022
SC	97	0.775	0.0193	0.023
GA97	49	0.817	0.0349	0.023
GF96	17	0.882	0.0468	0.028
GF97	58	0.800	0.0231	0.025
GF	74	0.812	0.0209	0.026
Total Atlantic	302	0.795	0.0117	0.023
Total Gulf	74	0.812	0.0209	0.026

The distribution of ND4 haplotypes among geographic samples was similar (Table 2). All five major haplotypes were represented at each location, and all haplotypes that occurred in two or more individuals were represented in at least two geographic samples. In tests for population structure based on allele frequency, significant heterogeneity was not detected over temporally pooled Atlantic samples ( $P = 0.34$ ), between Atlantic and Gulf samples ( $P = 0.30$ ), or in any pairwise comparison of geographic samples (Table 4). Similarly, analyses of population structure in which haplotype relatedness was considered ( $\Phi_{ST}$ ) were nonsignificant at all hierarchical levels.



**Fig. 2** *S. maculatus*. Gene diversity ( $H_E$ ) analysis for ND4 data. Shown are 95% confidence belt (dashes) and plotted data for each sample (black squares). Sample abbreviations follow Table 1. For each sample size, the original data from all samples was pooled, 1,000 permuted resamples were drawn of indicated size, and for each resample,  $h$  was calculated. Then 95% confidence intervals for  $h$  were calculated from the 1,000 values

**Table 4** *Scomberomorus maculatus*. Probability of homogeneity in allele frequency for the ND4 locus. Pairwise comparisons of geographic samples pooled over years. Probabilities from  $\Phi_{ST}$  analyses are shown above diagonal (divergence including haplotype relatedness), exact probability test results below (divergence in allele frequency only). Sample abbreviations follow Table 1

	CB	NC	SC	GA	GF
CB	—	0.74	0.22	0.86	0.37
NC	0.57	—	0.53	0.65	0.42
SC	0.24	0.23	—	0.31	0.82
GA	0.47	0.80	0.14	—	0.52
GF	0.33	0.69	0.76	0.69	—

## Actin intron locus

We verified the identity of the actin intron product and detected intra-specific polymorphism. BLAST (version 2.0) searches of the GenBank database for a match of the partial actin exon sequences to published sequences resulted in over 200 highly significant matches to actin exon, predominantly the  $\alpha$  isoform (probability that match was random  $P < 0.0001$ ; Altschul et al. 1990). Restriction enzymes *HhaI* and *RsaI* revealed polymorphisms in the actin intron. *HhaI* was chosen for further analysis because its simple restriction pattern (one fixed and one variable site) facilitated scoring of heterozygous individuals. The actin intron was amplified and digested with *HhaI* for 379 YOY Spanish mackerel (Table 5). Two alleles were detected and the distribution of genotypes conformed to Hardy–Weinberg expectations in all samples. Gene diversities and allele frequencies are presented in Table 5.

Comparable levels of nuclear gene diversity were found among both temporal and geographic samples. Two alleles were present in all samples. Gene diversities ranged from 0.114 to 0.318 among unpooled samples, and 0.158 to 0.258 among temporally pooled samples (mean  $H_E = 0.219$ ). As with ND4,  $H_E$  values for all samples fell within expected 95% confidence intervals (not shown) and were within two standard errors of each other, indicating that the level of variation did not differ significantly among samples (Table 3).

Temporal analyses revealed little variation in allele frequencies between samples of YOY Spanish mackerel taken over two or more years at the same location (Table 5). No significant differences were found in the distribution of allele frequencies among year classes from Chesapeake Bay ( $P = 0.87$ ), South Carolina ( $P = 0.11$ ), and Gulf ( $P = 1.00$ ). Therefore, temporal samples for each geographic location were pooled for subsequent analyses.

**Table 5** *Scomberomorus maculatus*. Sample size for actin intron ( $2n$ , twice the number of individuals surveyed for diploid loci), gene diversity ( $H_E$ ), standard error of  $H_E$  (Nei 1987), frequency of the most common allele resulting from digestion with *HhaI* (A), and its 95% bootstrap confidence interval (CI)

Sample	$2n$	$H_E$	Standard error	A	CI
CB95	34	0.114	0.0714	0.941	0.074
CB96	96	0.189	0.0494	0.896	0.063
CB97	106	0.188	0.0470	0.896	0.057
CB	236	0.177	0.0311	0.903	0.038
NC96	82	0.158	0.0512	0.915	0.061
SC96	92	0.318	0.0506	0.804	0.082
SC97	100	0.198	0.0489	0.890	0.060
SC	192	0.258	0.0361	0.849	0.051
GA97	100	0.258	0.0501	0.850	0.070
GF	148	0.242	0.0411	0.870	0.057
Total Atlantic	610	0.214	0.0200	0.879	0.025
Total Gulf	148	0.242	0.0411	0.870	0.057

**Table 6** *Scomberomorus maculatus*. Probability of homogeneity in allele frequencies for the actin intron locus. Abbreviations follow Table 1. Pairwise comparisons of allele frequencies from digestions with *HhaI* are shown

	CB	NC	SC	GA	GF
CB	–				
NC	0.83	–			
SC	0.10	0.17	–		
GA	0.19	0.25	1.00	–	
GF	0.25	0.29	0.88	0.85	–

Allele frequencies were also similar among the geographic locations. No significant heterogeneity was demonstrated among Atlantic collection locations, or in pairwise comparisons of all collection locations (Table 6). When the Atlantic samples were combined and compared with the Gulf samples, the probability of homogeneity was 0.58.

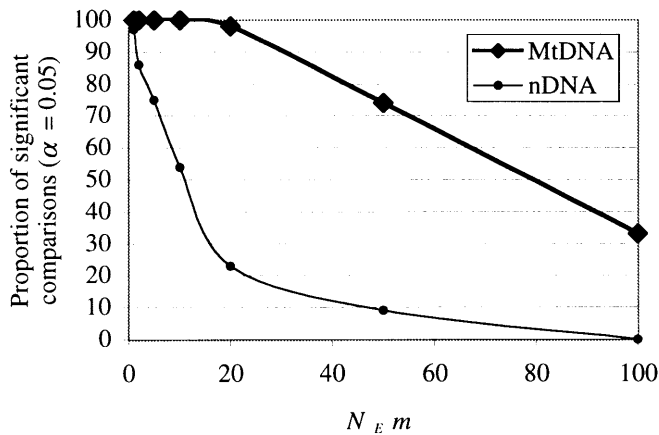
Combining the information from the mitochondrial and nuclear loci provided no evidence for significant temporal variation in the Chesapeake Bay ( $P = 0.97$ ), South Carolina ( $P = 0.19$ ), or Gulf ( $P = 0.37$ ). Similarly, the combined data sets did not provide indication of geographic heterogeneity among the Atlantic collections ( $P = 0.24$ ), or between the Atlantic and Gulf collections ( $P = 0.47$ ). None of the  $F_{ST}$  and  $\Phi_{ST}$  values were significantly greater than zero, indicating a lack of population heterogeneity.

## Power analysis

Considering mode of inheritance, marker diversity, and sample sizes (Atlantic vs. Gulf), statistical power was greater in mtDNA analysis than in nDNA analysis, despite a two-fold lower mtDNA sample size (Fig. 3). For actin we estimated an 80% probability of detecting significant divergence between equilibrium populations exchanging less than a single individual (two nuclear alleles) per generation. However, for ND4 we estimated a greater than 90% probability of detecting significant divergence between equilibrium populations exchanging less than 20 individuals (10 mitochondrial molecules) per generation.

## Discussion

Based on analysis of mitochondrial ND4 and nuclear actin intron loci, we cannot disprove the null hypothesis that Spanish mackerel comprise a single, genetically homogeneous population. Nor can we reject the null hypothesis that YOY Spanish mackerel of different year classes at the same location are genetically homogeneous. Lack of significant genetic divergence may be attributable to a lack of statistical power to detect a true difference, or to genetic homogeneity among putative spawning stocks.



**Fig. 3** Computer simulation sensitivity analysis for Atlantic versus Gulf allele frequency heterogeneity comparisons. Two populations under mutation, migration, and genetic drift equilibrium were simulated for a range of migration rates. The proportion of significant population comparisons was determined from 100 replicates, with significance determined using Roff and Bentzen (1989) chi-square analysis. MtDNA analyses (*dark line*) were calculated with haplotypic diversity levels averaging 0.8 and with sample sizes of 75 versus 300. Nuclear analyses (*thin line*) were calculated with heterozygosity levels averaging 0.3 and with sample sizes of 150 versus 600.  $N_E m$  refers to the product of effective population size ( $N_E$ ) and migration rate ( $m$ )

The sample design in this study was sufficient for a sensitive genetic analysis of population divergence. The probability of detecting population divergence is a function of the amount of divergence between samples (effect size) and sample size. The level of genetic divergence was maximized by analyzing YOY samples, choosing moderately polymorphic loci, and including a mitochondrial locus. Distinct spawning stocks may co-occur as adults at over-wintering grounds or along migratory pathways and subsequently separate into distinct and cohesive spawning groups due to natal site fidelity. Sampling YOY minimizes potential mixing of putatively distinct spawning stocks by sampling the progeny of spawning stocks before they have migrated. An intermediate mutation rate is generally considered a good tradeoff between sensitivity to recent divergence and necessity of large sample size to describe adequately the frequency of each allele class for chi-square analysis. To maximize statistical power in an analysis of population structure, Stepien (1999) considered only the 20 most common alleles, resulting in a level of diversity comparable to that of the present study. Finally, the four-fold lower effective size of mitochondrial over nuclear markers enhances the effect of genetic drift and may result in increased genetic divergence between subdivided populations.

An additional advantage of our analysis was that it was buffered from sampling error by repeated sampling of geographic locations. A finding of statistically significant genetic divergence based on samples taken from a single point in time and space may be compromised by unknown sampling biases. Spanish mackerel live for up to 9 years, but most individuals in the population are

only 1–3 years old (Powell 1975). Spanish mackerel population size also fluctuates greatly over periods of 2–4 years (Chittenden et al. 1993). Given a short life span and unstable demographics, allele frequencies may fluctuate significantly from year to year. Sampling of individuals over time largely circumvents this sampling bias (Waples 1998).

The genetic homogeneity detected among YOY Spanish mackerel surveyed in this study is consistent with results of the large-scale allozyme study of adult Spanish mackerel by Nakamura (1987) but contrasts with the hemoglobin study of Skow and Chittenden (1981) and morphological study of Collette and Russo (1984). Different results among studies may relate to differences in sample sizes and phenotypic expression of the latter studies. Both of the studies that reported differences among collections of Spanish mackerel employed samples sizes much smaller than the two studies that reported homogeneity and were, therefore, more susceptible to sampling error. The differences found by Skow and Chittenden (1981) and Collette and Russo (1984) require confirmation on a large scale. Furthermore, the two studies that reported differences also analyzed phenotypic characters (morphology, hemoglobin banding patterns) that in some fishes are known to be influenced by environmental conditions (Barlow 1961; Clementi et al. 1994).

The preponderance of evidence suggests that western Atlantic and Gulf migratory groups of Spanish mackerel are not genetically distinct. Natal site fidelity in Spanish mackerel either does not exist or is not strong enough to offset genetic mixing of individuals spawned from either different locations along the Atlantic coast or between the Atlantic and Gulf. This result supports the assumed highly migratory nature of this species. The same group of fish apparently migrates up the western Atlantic coast in spring, spawning when conditions are appropriate. If mixing were incomplete, one might also expect genetic diversity to decrease with latitude as different stocks stopped along the coast during the northern migration. This pattern was not observed. The diversity of the northernmost sample (Chesapeake Bay) did not differ from those of samples taken from throughout the range of the species.

Spanish mackerel revealed less population subdivision than detected in most other marine fishes whose range encompasses the Gulf of Mexico and western Atlantic. Gold and Richardson (1998) reviewed studies of mitochondrial variation in four fishes that were distributed in both the western Atlantic and Gulf: two estuarine-dependent (spotted sea trout *Cynoscion regalis*, red drum *Sciaenops ocellatus*), one reef-associated (greater amberjack *Seriola dumerili*), and one coastal pelagic species (king mackerel *Scomberomorus cavalla*). Evidence for population subdivision between Atlantic and Gulf samples was strong for the estuarine species and weak, but significant, for greater amberjack and king mackerel. Significant Gulf versus western Atlantic divergence was also reported for black sea bass

(*Centropristis striata*), menhaden (*Brevoortia* spp; Bowen and Avise 1990), striped bass (*Morone saxatilis*; Diaz et al. 1997), and Atlantic croaker (*Micropogonias undulatus*; Lankford et al. 1999). Genetic homogeneity between Atlantic and Gulf samples, however, was reported for a reef-associated species (vermillion snapper *Rhomboplites aurorubens*; Bagley et al. 1999) and for several sharks (Heist et al. 1995, 1996; Heist and Gold 1999). For the reef-associated species dispersal was thought to occur at pelagic egg and larval stages, for king mackerel at all stages, and for sharks as adults. For both king and Spanish mackerel, stock mixing is likely facilitated during the adult life history stage when Gulf and Atlantic individuals co-occur in over-wintering grounds off southern Florida, although mixing may be more pronounced in Spanish mackerel.

From an ecological and fisheries management perspective, even a sensitive genetic analysis is not sufficient to determine that there is no difference among putative stocks. Migration on the order of tens of individuals per generation is sufficient to homogenize allele frequencies among genetic stocks for both markers. While sufficient to homogenize allele frequencies, this level of migration may still be too low for healthy stocks from one area to replenish depleted stocks rapidly from another area of the range.

The results from this study support the hypothesis that seasonal abundance of Spanish mackerel along the Gulf and western Atlantic coasts is regulated, in part, by Florida's winter fishing practices. Abundance of Spanish mackerel has exhibited large variation in time and space over the past 150 years (Chittenden et al. 1993). Chittenden et al. (1993) hypothesized that a recent pulse of high catch in the Chesapeake Bay may be explained by good recruitment in the region, or greater escapement from the Florida gill net fishery. In that our results suggest that Spanish mackerel comprise a single, genetically homogeneous unit, management measures implemented in one region may have broad effects.

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