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Microsatellite variation suggests substantial gene flow between king mackerel (*Scomberomorus cavalla*) in the western Atlantic Ocean and Gulf of Mexico

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Abstract

We developed microsatellite loci for king mackerel (*Scomberomorus cavalla*) and used them to investigate population structure and gene flow between samples from regional localities in the western Atlantic Ocean (Atlantic) and Gulf of Mexico (Gulf). Based on mark-recapture and spatial–temporal sampling, king mackerel in US waters have been perceived as organized into two or more ''migratory units'' that tend to move in the spring from southern wintering areas off south Florida and the Yucatan peninsula to more northern spawning areas along the southeastern US coast and the northern Gulf. We surveyed allelic variation at five microsatellite loci between samples of king mackerel from eight geographic localities in the Atlantic and Gulf. Tests of homogeneity in allele distribution indicated that two samples from the northern Gulf (Port Aransas, TX, and Gulfport, MS) differed significantly from the remaining samples. However, no significant genetic differences were found between samples representing geographic extremes, and no significant geographic patterns of genetic divergence were found when samples were combined into regional groupings reflecting current hypotheses of population structure. There also was no evidence of an isolation-by-distance effect. We hypothesize that the regional migratory groupings in king mackerel do not restrict gene flow to the extent that significant genetic population structure may arise.

Keywords: Microsatellites; Gene flow; Population structure; King mackerel; *Scomberomorus cavalla*

1. Introduction

King mackerel (*Scomberomorus cavalla*) are coastal pelagic fish (family Scombridae) found in the western Atlantic Ocean, from the Gulf of Maine to Brazil, and throughout most of the Gulf of Mexico and Caribbean Sea (Collette and Nauen, 1983). Mature fish are large (up to 1.7 m and 45 kg) and appear to be capable of long-distance dispersal. Indeed, king mackerel migrate seasonally from wintering areas off southern Florida and the Yucatan peninsula to spring and summer spawning grounds along the Atlantic coast (Atlantic) of the United States and the northern Gulf of Mexico (Gulf). However, population studies have suggested that there is little movement of individuals between the Atlantic and Gulf (e.g., Schaefer and Fable, 1994). Coincident with this observation is an apparent biogeographic boundary

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associated with the Florida peninsula and Florida Keys archipelago. Briefly, patterns of variation in mitochondrial (mt) DNA between a number of coastal, marine, or anadromous species have revealed either deep phyletic branches or significant differences in haplotype (allele) frequencies that distinguish clades or groups from the Atlantic or Gulf (Avise, 1992; Gold and Richardson, 1998). Minimally, these patterns of mtDNA variation suggest that dispersal between these regions has been restricted historically. However, whether the biogeographic boundary associated with the Florida peninsula affects (or has affected) dispersal among highly vagile, pelagic species such as king mackerel remains to be tested rigorously.

Based on extensive mark-recapture (Schaefer and Fable, 1994; Fable et al., 1987; Sutter et al., 1991) and temporal/geographic sampling studies (Collins and Stender, 1987; Trent et al., 1983; Grimes et al., 1990), it has been hypothesized that king mackerel in US waters are comprised of distinct populations in the Atlantic and Gulf. There is evidence that king mackerel in the two regions differ in rates of growth (DeVries and Grimes, 1997) and based on allozyme and other evidence, it has been suggested (DeVries and Grimes, 1997; Johnson et al., 1994) that the Gulf may contain two populations of king mackerel. Considering all of the data acquired to date, DeVries and Grimes (1997) suggested there might be three migratory units (populations) of king mackerel: one from the Atlantic, one from the eastern Gulf and one from the western Gulf.

Despite apparent differences in patterns of migration and growth rates, previous studies of genetic markers are ambiguous and have not demonstrated unequivocally whether king mackerel in US waters are subdivided genetically. Johnson et al. (1994) surveyed 50 allozyme loci among nearly 3500 fish and found only one locus (PEPA-2) that was distributed heterogeneously. Gold et al. (1997) examined 678 king mackerel from throughout the region, and confirmed the occurrence of heterogeneity at PEPA-2. They did not, however, find significant heterogeneity in frequencies of mtDNA haplotypes between samples from the Atlantic and the Gulf, nor did they find any spatial or clinal pattern to mtDNA variation within the Gulf. Thus, the issue of whether there is restriction of gene flow between regions, or whether intrinsic behavioral differences contribute to migratory patterns, remains unclear.

Extensive human exploitation has resulted in declining abundance of king mackerel, emphasizing the need for conservation efforts to maintain population numbers and naturally occurring genetic variation. Although our understanding of king mackerel population structure is incomplete, current management of king mackerel fisheries in US waters is based on distinction between fish in the Atlantic vs. those in the Gulf (GMFMC, 1985). To further investigate the distribution of genetic variation and to track potential gene flow in king mackerel, we developed microsatellite markers and analyzed allelic variation among eight geographic samples from the Atlantic and Gulf. Microsatellite markers have proven to be useful indicators of population structure in a variety of eukaryotic taxa including fishes (Tautz and Schlotterer, 1994; Jarne and Lagoda, 1996; O'Connell and Wright, 1997; Ruzzante et al., 1996, 1998). Because alleles at microsatellite loci arise and are dispersed for relatively short periods before being altered by subsequent mutation, the spatial distribution of alleles in a population may reflect short-term gene flow (O'Connell and Slatkin, 1993). Indeed, a majority of population studies that have employed microsatellites have reported significant genetic divergence, even among proximal geographic groups (Angers et al., 1995; Bentzen et al., 1996; McConnell et al., 1997; Ruzzante et al., 1998; but see Herbinger et al., 1997). Microsatellites, therefore, can reveal population subdivision at smaller spatial and temporal scales than possible with other commonly used genetic markers such as allozymes and mtDNA (Ruzzante et al., 1996) and identify divergence among king mackerel populations that has previously gone undetected. A finding of significant heterogeneity in microsatellite allele distributions between samples from the Atlantic and Gulf would support the hypothesis that king mackerel tend to migrate and spawn in discrete groups and that dispersal may be influenced by the unique geography, climate, or habitat associated with the Florida peninsula. Heterogeneity also might be detected between or among other localities, e.g., the eastern vs. western Gulf. Alternatively, spatial homogeneity of allele frequencies at microsatellite loci would be consistent the hypothesis that contemporary mixing of individuals occurs at a rate sufficient to prevent genetic divergence.

2. Materials and methods

2.1. Materials examined

Tissue samples were obtained from tournaments or charter boat catches at eight localities in the Atlantic and Gulf (Fig. 1). Sample localities (identification symbol and number of individuals) were: Morehead City, NC (NC; 45); Daytona Beach, FL (DB; 42); Key West, FL (KY; 41); Panama City, FL (PC; 44); Gulfport, MS (GP; 45); Galveston, TX (GV; 46); Port Aransas, TX (PA; 42); and Veracruz, Mexico (VC; 48). Tissue samples (usually heart) were stored in liquid nitrogen for transport to the laboratory, where they were stored at −80°C. Sex of individuals was recorded for samples from NC, KY, PC, GP, and VC. Otoliths were removed and sent to the US National Marine Fisheries Service (NMFS) laboratory in Panama City, FL, for otolith increment analysis (age determination). Genomic DNAs were isolated by standard phenol–chloroform extraction and ethanol precipitation methods. Samples included the same individuals examined by Gold et al. (1997) in their study of variation in mtDNA and PEPA-2. This permitted direct comparison of genotypes at microsatellite loci with those at PEPA-2.

2.2. Microsatellite development

Microsatellite development followed methods described in Broughton and Gold (1997). Genomic DNA from a single king mackerel from Key West, FL,

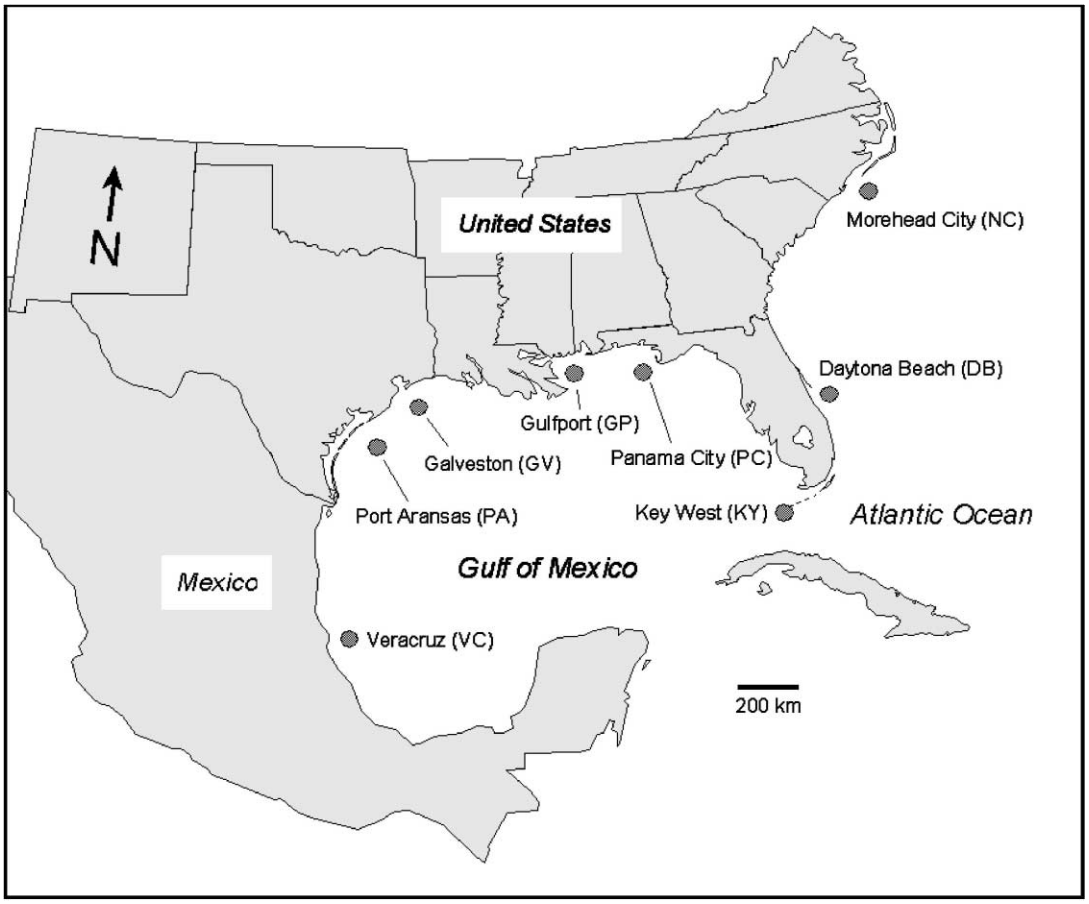

Fig. 1. Map of collection localities of king mackerel (*S. cavalla*) in the western Atlantic Ocean and Gulf of Mexico.

was used for cloning. *Dpn*II restriction fragments in the range 200–600 base pairs (bp) were ligated into the pGEM vector (Promega) and transformed into competent *E. coli* DH5α cells. Insert-containing colonies were grown in 96-well microtitre plates and transferred to a single (8 cm × 12 cm) nylon membrane using a robotic work station (Biomek, 2000, Beckman). Colonies were probed simultaneously with five ^{32}P-labeled repeat oligonucleotides [$(CA)_{15}$, $(GA)_{15}$, $(CCT)_7$, $(ATT)_7$, $(GACA)_8$] at 48°C. Nucleotide sequences of putative microsatellite-containing clones were determined with an Applied Biosystems 377 automated sequencer. Sequences confirmed the presence and identity of simple-sequence repeats and provided flanking sequences for primer design. Primers were designed with the aid of the computer program OLIGO. Primers sets were optimized by adjusting annealing temperature and magnesium ion concentration in amplification reactions.

Microsatellite amplification reactions contained approximately 5 ng of genomic DNA, 0.1 unit *Taq* DNA polymerase, 0.5 μM of each primer (one labeled with ^{32}P), 800 μM dNTPs, 1–2 mM magnesium ion, 1× *Taq* buffer pH 9.0 (Promega), and sterile deionized water in 10 μl volumes. Thermal cycling was conducted in 96-well plates with the following parameters: denaturation 94°C, 30 s; annealing (55–65°C), 30 s; and polymerization 72°C, 30 s. Allele sizes were determined by denaturing polyacrylamide gel electrophoresis and autoradiography with the sequenced clones as size standards.

2.3. Data analysis

Exact tests of Hardy–Weinberg proportions for each locus and of genotypic equilibrium between pairs of loci within and among samples were performed using the Markov-chain randomization approach (Guo and Thompson, 1992). Exact tests were also used to test for independence of the distribution of genotypes at each microsatellite locus with sex, age, and PEPA-2 genotype of individuals. Significance of *P*-values was tested by non-parametric permutation (10,000 replicates). To avoid small cell sizes in tests of independence, rare alleles were ''binned'' into neighboring size classes until each cell contained at least ten alleles. Allele classes were combined in the direction of the median allele size for each locus such that large alleles were combined into the next smaller class and small alleles were combined into the next larger class. Individual ages also were binned into the following classes: 1–3, 4–6, 7–9, and >10 years. We also tested for association (independence) between age and sex of individuals. All statistical tests involving multiple comparisons employed sequential Bonferroni correction for the critical level of α (Rice, 1989).

The eight geographic samples were combined into regional groupings designed to test current hypotheses of population structure in king mackerel. These hypotheses included the following: (i) a two-population hypothesis (one in the Atlantic and one in the Gulf) used in current management planning for the US king mackerel resource (GMFMC, 1985) and based primarily on mark-recapture data; (ii) a two-population hypothesis (one in the Atlantic plus eastern Gulf and one in the western Gulf) based largely on frequencies of alleles at the PEPA-2 locus (Johnson et al., 1994); and (iii) a three-population hypothesis (one in the Atlantic, one in the eastern Gulf, and one in the western Gulf) based on several lines of evidence (DeVries and Grimes, 1997). Because the Florida Keys lie at the boundary between the Atlantic and Gulf (and may be inhabited by different populations of king mackerel at different times of the year), separate tests were conducted with the sample from the Florida Keys (KY) included in either (but not both) Atlantic or Gulf groupings. It is worth noting, however, that the KY collection was made during late winter and early spring when fish from the Gulf migratory group are thought to occupy the area around the Florida Keys (Schaefer and Fable, 1994; Sutter et al., 1991). Samples were combined to represent the various population hypotheses as follows: (i) Atlantic (NC, DB [KY]) vs. Gulf ([KY], PC, GP, GV, PA, VC); (ii) Atlantic plus east Gulf (NC, DB, KY, PC) vs. west Gulf (GP, GV, PA, VC), and (iii) Atlantic (NC, DB [KY]) vs. east Gulf ([KY], PC) vs. west Gulf (GP, GV, PA, VC).

Tests of homogeneity in allele distributions employed estimates of hierarchical $F_{ST}(\theta)$ statistics (Weir and Cockerham, 1984) as implemented by the computer program GDA (Lewis and Zaykin, 2000). θ_P describes the variance of allele frequencies among regional groupings (populations) relative to the total variance, while θ_S describes the variance among individual samples within regional groupings. Bootstrapping across loci (1000 replicates) yielded 95%

confidence intervals that were used to test the null hypothesis that θ equaled zero. θ values (with bootstrap confidence intervals) also were calculated for 28 pairwise comparisons among individual samples. Homogeneity in allele distributions between pairs of samples for individual loci was examined with exact tests as implemented in genepop Version 3.1 (Raymond and Rousset, 1995a).

To examine the possibility of an isolation-by-distance effect, pairwise genetic distances between each of the eight samples in the form $\theta/(1-\theta)$ (negative values were changed to zero) were compared to physical distance. Physical distances (in kilometers) were measured either as the distance between sample localities following the coastline (approximating a linear stepping-stone model), or the natural logarithm (ln) of the most direct ocean distance between localities (approximating an island population model) (Rousset, 1997). The analysis employed the Mantel test, with 10,000 permutations to assess significance as implemented in GENEPOP.

3. Results

3.1. Development and characterization of king mackerel microsatellites

We screened 1152 insert-containing colonies and identified 55 putative microsatellite clones. Sequences of 12 inserts indicated the presence of a microsatellite locus. Of these, five microsatellite loci (*Sca*8, *Sca*30, *Sca*37, *Sca*44, and *Sca*47) were amplified reliably and used to assay genetic variation among the samples of king mackerel. Primer pairs and sequences of repeat motifs are provided in Table 1. The five loci were CA-rich and exhibited substantial allelic variation (Table 1). One locus (*Sca*30) contained contiguous runs of $(GA)_n$ and $(CA)_n$; while another (*Sca*44) was a tetranucleotide repeat $(GACA)_n$. For locus *Sca*44, seven of the 625 alleles assayed were intermediate relative to predicted sizes for a tetrameric repeat (corresponding to $8\frac{1}{2}$ or $10\frac{1}{2}$ repeats). Allele distributions for each locus by sample locality are given in Appendix A and Table 1. Genotypes at *Sca*8 and *Sca*47 could not be scored unambiguously in all samples (due possibly to degradation of DNA), and were treated as missing data in the analysis. Despite this missing data, each locus was represented by at least one population in each geographic group, thus all five loci were informative with respect to hypotheses of population structure. Allele sizes at *Sca*8, *Sca*44, and *Sca*47 were distributed more-or-less normally, while locus *Sca*30 exhibited a high frequency of one allele. Allele sizes at *Sca*37 were distinctly bimodal although this is not necessarily unexpected under standard population models (Palsbøll et al., 1999). There were no obvious differences in distribution of allele sizes among samples at any locus.

Table 1
Characteristics of five microsatellite loci from king mackerel

Locus	Repeat type	Number of alleles	Median allele size (number of repeats)	Primer sequences (5′–3′)
*Sca*8	CA	24	13	TCAGCTGTTCATTCCCATAGCCCA ATGAAGGAACAATGAGCCTCCAGC
*Sca*30	GA/CA[a]	20	23	TGGCTGTCGGTCACTCTGCCTC ACACACACGGGTACACACAGGG
*Sca*37	CA[b]	7	15	GCGCCGTGACTTTTTATTGCTC CAACAATTAGTCGCAGCCCTAG
*Sca*44	GACA	10	9	ATGGCCAAATGGCACATAATCA GGGCAGCTCCATGGGTCTGAGT
*Sca*47	CA	22	17	TCAAAGAGTGAAGCAGGTATTC GGGATCATGCAGCAAGGTAACA

[a] Compound repeat.
[b] Contains one TA imperfection.

Table 2
Summary statistics for each microsatellite locus in each sample of king mackerel[a]

Locus	Sample							
	NC	DB	KY	PC	GP	GV	PA	VC
*Sca*8								
n_i/n_a	NA	42/84	41/80	NA	44/88	NA	35/65	46/90
H_0	NA	0.93	0.95	NA	0.87	NA	0.97	0.91
P_{HW}	NA	0.989	1.0	NA	0.999	NA	0.999	0.999
*Sca*30								
n_i/n_a	45/90	42/82	41/81	41/80	37/69	44/86	40/79	48/94
H_0	0.87	0.75	0.75	0.62	0.78	0.69	0.82	0.67
P_{HW}	0.999	0.999	1.0	0.999	1.0	1.0	0.999	1.0
*Sca*37								
n_i/n_a	43/86	42/84	41/82	24/48	45/90	46/91	41/81	48/96
H_0	0.58	0.50	0.59	0.50	0.49	0.60	0.45	0.33
P_{HW}	0.317	0.279	0.913	0.043	0.649	0.073	0.329	0.527
*Sca*44								
n_i/n_a	43/85	41/80	41/82	43/86	43/86	34/62	28/56	44/88
H_0	0.57	0.69	0.56	0.47	0.81	0.61	0.50	0.64
P_{HW}	0.394	0.522	0.046	0.456	0.461	0.710	0.100	0.662
*Sca*47								
n_i/n_a	40/67	38/68	41/81	42/75	42/75	NA	36/71	46/92
H_0	0.96	0.90	0.93	0.76	0.94	NA	0.87	0.78
P_{HW}	0.754	0.945	0.999	0.755	0.733	NA	0.574	0.976

[a] Sample localities are identified as in Section 2. n_i: number of individuals scored; n_a: number of alleles scored; H_0: observed heterozygosity; P_{HW}: probability of conforming to expected Hardy–Weinberg proportions; NA: data not available.

Summary statistics for each locus in each sample are listed in Table 2. Observed heterozygosities over all loci and samples ranged from 0.47 to 0.96. Frequencies of homozygotes and heterozygotes closely matched expected proportions under Hardy–Weinberg equilibrium, although one locus (*Sca*37), in one sample (PC), exhibited heterozygote deficiency, while another (*Sca*44 in KY) exhibited heterozygote excess (neither deviated significantly from equilibrium expectations after Bonferroni correction). For some individuals a single allele pattern that appeared faint on the autoradiograph (relative to other individuals for the same locus and the same individual for other loci) was observed. Considering the possibility of null alleles, we did not assume these were homozygotes, rather only a single allele was scored for those individuals; hence the discrepancy between the number of alleles and number of individuals in Table 2. Such cases were excluded from Hardy–Weinberg tests. Genotypes between pairs of microsatellite loci among samples were distributed randomly except for a non-random association of genotypes at *Sca*37 and *Sca*44 (Table 3). The probability value for this comparison, however, was non-significant following Bonferroni correction. In addition, the association between *Sca*37 and *Sca*44 appeared to be unduly influenced by one sample (DB), where the probability of random distribution of genotypes at the two loci was 0.007. Following Bonferroni corrections, genotypes at the five microsatellite loci also were independent of the

Table 3
Probability of genotypic equilibrium among loci for combined samples[a]

Locus	*Sca*8	*Sca*30	*Sca*37	*Sca*44
*Sca*30	0.314			
*Sca*37	0.996	0.321		
*Sca*44	0.412	0.349	0.024*	
*Sca*47	0.190	0.858	0.161	0.207

[a] *P*-values of exact tests.
* Non-significant when corrected for multiple tests.

Table 4
Probability of independence of genotypes at microsatellite loci with age, sex, and genotypes at PEPA-2[a]

	*Sca*8	*Sca*30	*Sca*37	*Sca*44	*Sca*47
Age	0.907	0.261	0.990	0.132	0.977
Sex	0.494	0.032[*]	0.895	0.168	0.266
PEPA-2	0.513	0.547	0.916	0.293	0.990

[a] *P*-values of exact tests.
[*] Non-significant when corrected for multiple tests.

sex and age of individuals and of genotypes at PEPA-2 (Table 4). An exact test for association of age and sex of individuals revealed independence ($P = 0.188$) of these two parameters also.

3.2. Analysis of microsatellite allele frequencies

For tests of homogeneity in allele distributions we present only estimates of $F_{ST}(\theta)$; results of comparable analyses employing R_{ST} (Slatkin, 1995) and exact tests (Raymond and Rousset, 1995b) did not differ qualitatively from θ values reported (Table 5). For all tests of hypotheses of regional population structure, allele distributions were homogeneous as indicated by confidence intervals that overlapped zero (θ_P, Table 5). In contrast, confidence intervals for all θ_S (among the eight samples) did not include zero (Table 5), suggesting heterogeneity in allele frequencies among the eight samples. Pairwise comparisons indicated that significant differences in allele distributions primarily involved the samples PA, DB, and GP (Table 6). Exact tests among samples for individual loci (not shown) indicated that the deviations from homogeneity identified above were attributable almost entirely to two loci (*Sca*44 and *Sca*47) and generally were restricted to comparisons involving the samples PA and GP. The two analytical approaches thus identified PA and GP as having allele distributions that differed from each other and from the remaining samples. However, there was no apparent geographical pattern to the genetic divergence between samples, and there was little concordance among loci in patterns of geographic variation. Finally, the probability of a positive association between genetic distance and geographic distance (which measures an isolation-by-distance effect) was non-significant (θ-coastline distances, $P = 0.143$; θ-ln direct distances, $P = 0.127$).

Table 5
Tests of genetic differences among regional groups employing θ_P and θ_S and their respective 95% confidence intervals

Hypothesis	θ_P	95% CI	θ_S	95% CI
Gulf + KY vs. Atl	0.0014	−0.0024–0.0052	0.0101	0.0073–0.0141
Gulf vs. Atl + KY	0.0004	−0.0027–0.0034	0.0095	0.0054–0.0138
WGulf vs. EGulf + Atl	0.0024	−0.0001–0.0048	0.0103	0.0046–0.0161
WGulf vs. EGulf + KY vs. Atl	0.0014	−0.0014–0.0037	0.0097	0.0054–0.0144
WGulf vs. EGulf vs. Atl + KY	0.0012	−0.0008–0.0037	0.0103	0.0055–0.0159

Table 6
Values of θ for pairwise comparisons among samples

	VC	PA	GV	GP	PC	KY	DB
PA	0.0130[a]						
GV	0.0011	−0.0012					
GP	0.0018	0.0095[a]	0.0032				
PC	−0.0001	0.0086[a]	0.0059	0.0022			
KY	0.0033	0.0068[a]	0.0052	0.0002	0.0037[a]		
DB	0.0026[a]	0.0053[a]	0.0063[a]	0.0028[a]	0.0004	0.0026	
NC	0.0013	−0.0012	0.0043[a]	0.0058[a]	0.0068	0.0008	0.0005

[a] Values where the lower 95% confidence limit did not include zero.

4. Discussion

4.1. King mackerel microsatellites

We identified approximately 50 microsatellites in 1152 clones, indicating that about 4% of king mackerel genomic fragments in the 200–600 bp range contain a microsatellite. The five microsatellites used tended to be rich in CA repeats, and each exhibited substantial allelic variation. Within the samples, genotypes at each locus were in proportions expected under Hardy–Weinberg equilibrium. In addition, the five microsatellites appeared to vary independently of one another and with the sex, age, and PEPA-2 genotypes of the individuals assayed. These observations suggest that king mackerel microsatellites are similar to those in other teleost fishes and other vertebrates (O'Connell and Wright, 1997; Brooker et al., 1994; Bentzen et al., 1996).

4.2. Analysis of variation in allele distributions and population structure in king mackerel

None of the current hypotheses of geographic population structure in king mackerel were supported by the microsatellite data. While none of the θ_P values (Table 5) differed significantly from zero, it was worth examining allelic distribution among individual samples as these might reveal heterogeneity in a pattern that was not specifically examined or tested. It is not always easy to identify appropriate ''subpopulations'' between which restricted gene flow might be expected, thus selecting appropriate groups to test for homogeneity can be problematic (Rousset, 1997). However, the samples of king mackerel in this study did not appear to represent independent reproductive populations but rather to represent spatial–temporal samples of a larger reproductive group. If independent migratory groups existed, individual samples would be expected to reflect a broader population pattern. For example, one might expect VC (the sample from Veracruz, Mexico) or GV (Galveston, TX) to differ from DB or NC (samples from the US Atlantic coast), but not necessarily from neighboring samples. We found no consistent pattern of difference at any locus even between samples at the ends of the geographic sampling distribution.

We suspect at least some of the sample-pairwise differences observed (either as direct comparisons or as θ_S) were due to sampling effects. In Ruzzante's (1998) investigation of bias and sampling variance for F_{ST} (and several other measures) for microsatellite data, he concluded that sampling variances were relatively small for sample sizes ranging between 50 and 100 individuals or higher. Our sample sizes of roughly 45 individuals each are therefore in a range where sampling variance may be slightly higher and differences between a few samples are not unexpected. Alternatively, our tests of regional hypotheses involved grouping samples such that each region usually contained at least two samples ($\sim$90 individuals). Thus, sampling variance for these cases should be much less; a fact apparently reflected in the lack of significant differences between regional groups.

The reason(s) for the observed allele-frequency divergence in the samples from PA and GP could also be related to what has been termed ''chaotic patchiness'' (Hedgecock, 1994) or ''chaotic temporal variation'' (P. Bentzen, pers. comm.). In brief, genetic divergence among sampling years within a locality can be equal to or greater than genetic divergence among localities (Hedgecock, 1994; Smolenski et al., 1993; Purcell et al., 1996; P. Bentzen, pers. comm.). The mechanism(s) behind such temporal variation are not known, but Hedgecock (1994) suggested that it may be due to genetic drift brought about by differential reproductive success of a given cohort. This hypothesis would not seem tenable for our samples of king mackerel for at least two reasons. First, each of our samples contained individuals from several different cohorts; and second, variation at each microsatellite locus appeared to be independent of variation in age of individual fish. Possible temporal instability in allele frequencies could, however, stem from experimental sampling error. We are now investigating this in king mackerel by sampling several localities in Florida in different years.

Our analysis also failed to find association between microsatellite and PEPA-2 genotypes. Variation at PEPA-2 is distributed in a clinal fashion (the frequency of the fast allele ranged from less than 0.1 off the coast of North Carolina in the Atlantic to greater than 0.8 off the coasts of Texas and Mexico in the Gulf), with a sharp discontinuity in allele frequency occurring near the panhandle region of northwest Florida (Johnson et al., 1994; Gold et al., 1997). This pattern of variation at PEPA-2 is largely

the evidence for the two-population hypothesis of Johnson et al. (1994), and part of the evidence for the three-population hypothesis of DeVries and Grimes (1997). Neither microsatellites nor mtDNA exhibited a clinal pattern of variation either within or between the Atlantic and Gulf. PEPA-2 also appears to be unique among genetic markers examined to date in that genotypes appear to be distributed non-randomly with both age and sex of individuals (Gold et al., 1997). It is possible that variation at PEPA-2 is not the result of genetic drift in isolated populations, and hence may not reflect contemporaneous population structure. Mechanisms that might account for the variation at PEPA-2 include spatially and/or temporally varying selective differences among genotypes, ontogenetic or developmental differences in allele expression, or stochastic historical factors (e.g., where there might have been a heterogeneous distribution of alleles during range expansion, see below).

Analyses of population structure based on allele frequencies such as this assume that the population is at migration-drift equilibrium. If two populations of effective size N_e exchange a fraction of migrants m each generation, and the mutation rate is much lower than m, then M ($=2N_em$) will estimate the effective number of migrants at migration-drift equilibrium. Non-equilibrium conditions could result from recent range expansion and/or changes in effective population size. King mackerel may have expanded their range within the last 10–15,000 years, as northern parts of their current range experienced much cooler temperatures and lower sea levels during Wisconsin glaciation (Rezak et al., 1985). Consequently, king mackerel may have occupied more southern regions during glacial periods, and moved into the northern Gulf and up the Atlantic coast with climatic warming. Colonization of northern regions by independent groups from centralized refugia could result in an isolation-by-distance effect, provided migration among regions was limited. However, evidence from mark-recapture studies suggests that current exchange of individuals between the western Atlantic and Gulf ranges from 3 to 6% and that seasonal overlap of Atlantic and Gulf fish in waters off southeastern Florida may be as high as 41.8% (Sutter et al., 1991). Thus, even if the king mackerel population has not reached migration-drift equilibrium, gene flow still appears to be the primary factor affecting the distribution of allelic variation at microsatellite loci.

Interpretation of our failure to reject the null hypothesis of allele frequency homogeneity between regional groups depends on the confidence that can be placed on that result. To this end, we have estimated the power of our analysis for each locus following Baverstock and Moritz (1996, Table 1); cited from Richardson et al. (1986). Estimates of power were approximately 0.8, indicating β (=probability of a Type II error, or failing to reject the null when it is false) to be approximately 0.2 per locus. With five independent loci, the global β should be substantially less than this value. Thus, while it may be inappropriate to explicitly accept the null hypothesis, it does appear that our data set is robust.

Results of this study are consistent with the preponderance of data, both genetic and otherwise, suggesting a meaningful level of gene flow among king mackerel in US waters. The results are also consistent with the hypothesis that the Florida peninsula and Keys are not an effective impediment to movement of these pelagic fishes. Although many, broadly distributed pelagic fishes do not exhibit significant population structure within ocean basins (Graves, 1998), some species of mackerel (Begg et al., 1998; Nesbo et al., 2000) and herring (Shaw et al., 1999) do exhibit genetic differences that may be associated with specific spatial or temporal spawning regimes. Thus, if king mackerel exhibit significant fidelity to spawning sites in the Atlantic or Gulf, detection of consequent genetic divergence would be expected. The lack of such evidence suggests that there is effective movement of individuals between regions. Indeed, even at the low estimate of 3% of individuals exchanged per year, this level of gene flow is sufficient to prevent genetic divergence between populations (Slatkin, 1995). It is our opinion that current management planning that assumes two migratory units does not have a negative effect on the king mackerel resource, even though recognition of separate migratory units appears not to be supported by available genetic data.

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Appendix A

Allele frequencies for each microsatellite locus in each sample of king mackerel.

Locus alleles	Sample locality							
	NC	DB	KY	PC	GP	GV	PA	VC
*Sca*8								
3	–	0	0	–	0	–	0	1
4	–	2	3	–	0	–	1	1
5	–	0	1	–	0	–	1	0
6	–	5	7	–	7	–	1	4
7	–	5	3	–	6	–	11	4
8	–	0	0	–	1	–	1	0
9	–	1	1	–	0	–	0	0
10	–	3	4	–	2	–	0	2
11	–	15	7	–	9	–	6	9
12	–	9	19	–	15	–	5	14
13	–	10	15	–	20	–	13	19
14	–	6	3	–	6	–	9	11
15	–	2	1	–	1	–	0	0
16	–	0	1	–	1	–	3	2
17	–	1	0	–	0	–	1	0
18	–	0	0	–	1	–	0	0
19	–	6	4	–	6	–	4	5
20	–	9	6	–	6	–	1	8
21	–	2	1	–	3	–	0	2
22	–	0	1	–	0	–	3	21
23	–	8	3	–	2	–	5	5
24	–	0	0	–	0	–	0	1
25	–	0	0	–	1	–	0	0
26	–	0	0	–	1	–	0	0
*Sca*30								
22	3	4	3	1	2	1	2	2
23	36	38	40	50	36	48	37	48
24	3	1	1	3	1	2	1	2
25	3	7	5	4	3	6	3	1
26	1	1	4	3	4	2	2	4
27	6	2	4	4	2	1	3	7
28	7	3	2	1	3	3	4	6
29	3	3	2	3	1	3	4	3
30	1	2	2	0	2	5	1	1
31	10	3	6	4	4	4	7	6
32	10	9	3	3	4	6	6	3
33	1	1	2	1	3	3	1	0
34	1	5	1	2	1	1	2	2
35	2	0	2	1	1	0	4	2
36	3	1	2	0	1	0	2	3
37	0	2	2	0	0	1	0	0
38	0	0	0	0	1	0	0	0
40	0	0	0	0	0	0	0	1
41	0	0	0	0	0	0	0	1
42	0	0	0	0	0	0	0	1
43	0	0	0	0	0	0	0	1
*Sca*37								
14	0	1	1	0	2	1	0	0
15	55	61	50	36	59	48	45	64
16	0	1	0	0	2	4	5	0
17	0	0	1	0	0	1	0	2
20	30	19	28	12	26	34	31	28
21	1	2	2	0	1	3	0	1
22	0	0	0	0	0	0	0	1
*Sca*44								
4	1	1	0	0	0	0	0	0
5	0	0	0	0	0	0	1	0
6	5	8	0	3	4	6	7	0
7	2	1	2	2	6	1	1	1
8	33	31	38	34	29	17	17	27
8.5[a]	0	0	5	0	0	0	0	1
9	34	29	24	35	23	26	27	41

Appendix A (*Continued*)

Locus alleles	Sample locality							
	NC	DB	KY	PC	GP	GV	PA	VC
10	9	10	13	12	23	12	3	18
10.5[a]	1	0	0	0	0	0	0	0
11	0	0	0	0	1	0	0	0
*Sca*47								
5	0	0	0	0	0	–	0	2
9	0	0	1	0	0	–	0	0
10	0	0	1	0	1	–	0	0
11	1	0	1	0	0	–	0	4
12	7	6	5	7	3	–	0	3
13	11	12	12	13	1	–	0	16
14	11	10	11	14	7	–	4	17
15	3	4	5	0	15	–	6	2
16	3	1	4	3	5	–	5	3
17	10	5	7	10	4	–	18	9
18	5	10	6	4	6	–	11	3
19	3	6	11	7	5	–	13	9
20	5	2	5	0	4	–	4	8
21	5	7	8	11	8	–	7	9
22	1	2	2	2	5	–	0	2
23	0	0	0	0	4	–	1	0
24	0	1	0	0	6	–	1	2
25	2	2	0	2	0	–	0	1
26	0	0	0	2	0	–	1	0
27	0	0	2	0	0	–	0	1
32	0	0	0	0	1	–	0	0
35	0	0	0	0	0	–	0	1

[a] Alleles that differed by only two nucleotides at a tetranucleotide repeat locus.

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