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Mitochondrial DNA variation among red snapper (*Lutjanus campechanus*) from the Gulf of Mexico

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Abstract

Variation in mitochondrial DNA (mtDNA) was examined among 86 red snapper (*Lutjanus campechanus*) from three geographic localities in the northern Gulf of Mexico (Gulf). A total of 29 composite mtDNA genotypes (haplotypes) was found; one haplotype occurred in 39 of 86 (45.3%) individuals assayed, and 20 haplotypes occurred in only one individual each. Tests of heterogeneity in mtDNA haplotype frequencies among localities were not significant, and there was little evidence of phylogeographic structuring of mtDNA haplotypes. These findings are consistent with the hypothesis that red snapper in the northern Gulf comprise a single, panmictic population. The observed genetic homogeneity also indicates considerable gene flow (migration) among red snapper in the northern Gulf. Significant differences in levels of intrapopulational mtDNA variation were found among localities. Levels of intrapopulational mtDNA diversity in red snapper are low relative to other marine fish species studied to date.

Introduction

The red snapper (*Lutjanus campechanus*) is one of the most economically valuable fish species in the northern Gulf of Mexico (Gulf) (GMFMC, 1989, 1991). However, despite increased concern and management, red snapper production in the northern Gulf has declined steadily over the last 20 years; combined landings reached an all-time low between 1986 and 1988 (GMFMC, 1989; Goodyear and Phares, 1990). The decline in Gulf red snapper has been attributed to several factors, including mortality due to bottom-trawl fishing for shrimp (GMFMC, 1989; Goodyear and Phares, 1990). An additional cause for concern relative to the northern-Gulf red snapper fishery are recent estimates (Goodyear and Phares, 1990) which indicate that the spawning stock biomass per recruit (SSBR) is well below the 20% minimum required by the Gulf of Mexico Fishery Management Council to sustain the fishery. These estimates of SSBR indicate that the effective number of red snapper females may be low and that historical levels of genetic variability might be impacted severely. This process could potentially reduce the long-term adaptive potential of the species (Soulé, 1980; Frankel and Soulé, 1981).

Separate from the problem of decline in abundance is whether red snapper in the Gulf comprise a single unit stock or population. Currently, red snapper within the Gulf of Mexico Exclusive Economic Zone (EEZ) and adjoining Territorial Sea are considered a unit stock for management purposes (GMFMC, 1989, 1991). The prevailing view, however, is that both adult and juvenile red snapper are essentially nonmigratory, suggesting that separate breeding subpopulations or stocks might exist within the Gulf. This view is based largely on tagging studies and observations that juveniles and adults often exhibit substrate specificity (Bradley and Bryan, 1974; Beaumariage and Bullock, 1976; Fable, 1980).

In this study, we used restriction enzyme-site polymorphism of mitochondrial DNA (mtDNA) to determine levels of genetic variability among red snapper from 3 localities in the northern Gulf and to test the hypothesis that red snapper in the northern Gulf comprise a single, randomly mating population. Similar studies in a variety of organisms, including fishes, have shown that analysis of mtDNA is a powerful method for differentiating

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among subpopulations within species (Avise, 1987; Avise et al., 1987; Gold et al., 1993) and estimating levels of genetic variation among species (Avise, 1992; Richardson and Gold, 1993). Information on genetic stock structure would allow adjustment of fishery regulations by subregions should subregional stocks exist. Information on genetic variation is of interest relative to the concept that levels of genome-wide variation affect probabilities of population survival and fitness (Soulé 1980; Frankel and Soulé, 1981).

Results

Single digestions of mtDNA molecules from 86 red snapper surveyed with 13 restriction-endonuclease enzymes produced a total of 93 unique fragments (representing 70 restriction sites). The mean genome size of all single digestions (verified by mapping) was 16.8 ± 0.2 kb. No direct evidence for mtDNA size variation or heteroplasmy was observed among the individuals surveyed. Digestion patterns of the 13 enzymes revealed 29 composite mtDNA genotypes (haplotypes) across 3 geographic localities (Table 1). Haplotypes 1 and 10 were the most common; they occurred in 39 (45.3%) and 11 (12.8%) individuals, respectively. Twenty haplotypes were found in only one individual each. Estimates of percentage nucleotide sequence divergence among the 29 haplotypes ranged from 0.15 to 1.33 (mean ± SD = 0.50 ± 0.19).

Tests of heterogeneity in mtDNA haplotype frequencies among localities were not significant. Heterogeneity tests included a G test ($G_{[56]} = 59.1$; $p > 0.05$); a Monte Carlo randomization procedure using 1,000 bootstrapped replicates ($p = 0.851$); and *V* tests for the nine haplotypes found in more than one individual. All 9 *V* tests were not significant ($p > 0.05$).

MtDNA nucleon and intrapopulational nucleotide sequence (mtDNA) diversities varied among localities (Table 2). Nucleon diversities ranged from 0.626 (Port Aransas, TX) to 0.870 (Pensacola, FL), and intrapopulational mtDNA diversities ranged from 0.140 ± 0.008 (Port Aransas, TX) to 0.311 ± 0.012 (Pensacola, FL). Significant heterogeneity ($p < 0.05$) in intrapopulational mtDNA diversities was detected by single classification analysis of variance ($F_{[2, 1227]} = 70.37$). Mean separation using Duncan's multiple range test indicated that mean intrapopulational mtDNA diversity of the sample from Port Aransas, TX, was significantly ($p < 0.05$) lower than intrapopulational mtDNA diversities of the other 2 samples. MtDNA variation in red snapper, as estimated by both nucleon and intrapopulational mtDNA diversities, is among the lowest reported to date for marine fish species (Table 3).

Phenetic relationships (from UPGMA clustering) and parsimony networks of the 29 mtDNA haplotypes revealed little evidence of phylogeographic

Table 1 Distribution of 29 mtDNA composite digestion patterns (haplotypes) among samples of red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico.

Haplotype #	Composite MtDNA Genotype*	Locality† PSC	PFN	PAR
1	AAAAAAAAAAAAA	9	15	15
2	AEABAAAABAAAA	1	-	-
3	AABAAAAAAAAAA	1	1	2
4	AACABAAAAAAAA	1	-	1
5	AADAAAABAAAAA	1	-	-
6	BACAAAAAAAAAA	1	-	-
7	AADAAABABAAAA	1	-	-
8	AAAAACAAAABAA	1	-	-
9	ACAAAAAAAAAAA	1	-	-
10	AACAAAAAAAAAA	3	4	4
11	AAAAAACABAAAA	1	-	-
12	AAAAAAAAAABA	1	-	-
13	AAAAAAAAAABAA	1	-	1
14	AADAAAAAAAAAA	1	-	-
15	BAAAAAAAAAAAA	-	1	-
16	AAAAAAAABBAAA	-	1	-
17	AAAAAAABAAAAA	-	2	-
18	AAAAABAAAAAAA	-	1	1
19	AAAABAAAAAAAA	-	1	-
20	ABAAAAAAAAAAA	1	-	-
21	AAAAAAEAAABAA	-	1	-
22	AAEAAAAAAAAAB	-	1	-
23	AAEAAADBAAAAA	-	1	-
24	ADCAAAAAAAAAA	-	1	-
25	AADAAABAAAAAA	-	1	-
26	AACAAABAAAAAA	-	2	-
27	AAABAAAABAAAA	-	1	1
28	AAAAAAAAAACA	-	1	-
29	AAEAAAAAAAAAA	-	1	-
Totals		25	36	25

*Letters (from left to right) are digestion patterns (Appendix Table 1) for: *Apa*I, *Bcl*I, *Dra*I, *Hin*dIII, *Hpa*I, *Nco*I, *Nhe*I, *Pvu*II, *Sca*I, *Sma*I, *Sst*I, *Stu*I, and *Xba*I. †Locality abbreviations (acronyms) are: PSC (Pensacola, FL), PFN (Port Fourchon, LA), and PAR (Port Aransas, TX).

Table 2. MtDNA nucleon and intrapopulational nucleotide sequence diversities among red snapper from three geographic localities in the northern Gulf of Mexico. Sample sizes are given in parentheses.

Locality	No. of Haplotypes	Nucleon Diversity	Nucleotide Sequence Diversity (± SE)[a]
Pensacola, FL (25)	5	0.870	0.311 ± 0.012
Port Fourchon, LA (36)	17	0.821	0.282 ± 0.008
Port Aransas, TX (25)	7	0.626	0.140 ± 0.008

[a]Values are in percentages.

Table 3. Estimates of mtDNA variability in marine fishes.

Species	No. Individuals Surveyed	Nucleon Diversity	Intrapopulational Nucleotide Sequence Diversity (%)
Brevoortia tyrannus (Atlantic menhaden)[a]	17	1.00	3.19
Pomatomus saltatrix (Atlantic Bluefish)[b]	372	0.70	1.23
Clupea harengus (Atlantic herring)[c]	69	0.93	1.09
Sciaenops ocellatus (Red drum)[d]	693	0.95	0.57
Seriola dumerili (Greater amberjack)[e]	59	0.90	0.34
Lutjanus campechanus (Red snapper)[f]	86	0.78	0.25
Cynoscion regalis (Weakfish)[g]	370	0.13	0.13
Epinephelus morio (Red grouper)[e]	51	0.42	0.08

[a]Avise (1992).
[b]Graves et al. (1992b).
[c]Kornfield and Bogdanowicz (1987).
[d]Gold et al. (1993).
[e]Richardson and Gold (1993).
[f]This article.
[g]Graves et al. (1992a).

structuring (Figure 1). In the UPGMA phenogram (Figure 1A), all 29 haplotypes were joined at 0.77% nucleotide sequence divergence, and only 2 clusters of geographically cohesive haplotypes were found. Both clusters (one comprised of haplotypes 3, 22, and 29; one comprised of haplotypes 10, 24, and 26) contained a haplotype found in all 3 localities and 2 haplotypes found only among specimens from Port Fourchon, LA. The standard error of the most distant node, however, was greater than the distance between the first and last nodes, effectively collapsing all of the nodes. One of many

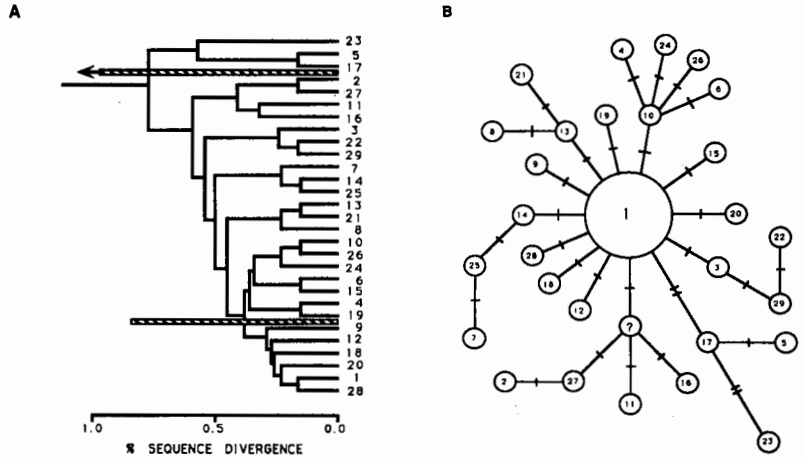

Figure 1. (A) UPGMA cluster analysis of percentage nucleotide sequence values. Operational units are the 29 composite mtDNA haplotypes found in red snapper. *Hatched bars* are standard errors of the nodes they overlie. (B) Parsimony network of 29 mtDNA haplotypes found in red snapper. Branches connecting haplotypes are drawn proportional to the number of restriction site changes (*hatch marks*) required to connect adjacent haplotypes. One haplotype (indicated by "?") was not found but was assumed to exist.

possible parsimony networks (Figure 1B) required a minimum of 31 steps, and one "assumed" haplotype (i.e., a haplotype not found in the survey). Haplotype 1 was considered central because it was the most common haplotype observed and because it invariably appeared as the "hub" in parsimony networks. Maximum parsimony analysis (using PAUP) of the 29 haplotypes generated 240 equally parsimonious trees. A strict consensus tree (not shown) revealed two clades: one comprised of haplotypes 8, 13, and 21; and one comprised of haplotypes 2, 11, 16, and 27. Neither clade is phylogeographically cohesive. The remaining 22 haplotypes were represented in the consensus tree as single lineages forming a large basal polytomy with the above 2 clades. In general, both phenetic and parsimony analyses indicated a high degree of genetic similarity among the 29 red snapper mtDNA haplotypes, with very little evidence of phylogeographic structuring.

Discussion

The absence of significant heterogeneity in mtDNA haplotype frequencies and the lack of mtDNA phylogeographic structure among samples from Florida, Louisiana, and Texas are consistent with the hypothesis that red snapper in the northern Gulf comprise a single, randomly mating population. These findings are in accord with current management policy (GMFMC, 1989, 1991) that red snapper in the northern Gulf comprise a unit stock.

The observed genetic homogeneity is also consistent with the hypothesis that gene flow (migration) among red snapper in the northern Gulf is sufficient to preclude significant genetic divergence across localities. This inference is not in accord with evidence that adult and juvenile red snapper are essentially sedentary, nonmigratory, and typically associated with specific substrates (Bradley and Bryan, 1974; Beaumariage and Bullock, 1976; Fable, 1980). It is known, however, that both adults and juveniles exhibit seasonal inshore-offshore movement in the Gulf and that adults are not always distributed over hard bottom or reef substrate (Bradley and Bryan, 1974; Beaumariage and Bullock 1976; Gutherz and Pellegrin, 1988). The genetic data indicate that red snapper also move laterally along the northern Gulf coast and that migrant individuals spawn in regions distant from their area of natal origin. Alternatively, the genetic data do not indicate the life-history stage in red snapper at which lateral movement occurs. Red snapper eggs and larvae are pelagic (Leis, 1987), and it is possible that significant red snapper migration occurs at the younger life-history stages. This would be important to know given the estimated mortality of age 0 to 1 red snapper caused by bottom-trawl fishing for shrimp (Goodyear and Phares, 1990; Nichols et al., 1990) and the effects of migration (gene flow), which reduce frequencies of individuals homozygous for deleterious recessive alleles and increase levels of genome-wide variation (Hartl and Clark, 1989).

There are several caveats to these observations. First, genetic homogeneity does not unequivocally establish the existence of a unit stock, but rather is simply consistent with the hypothesis that samples are drawn from a panmictic population. Proving the null hypothesis in this (or any similar) case is difficult to impossible. The second caveat regards the relatively low levels of mtDNA variation in red snapper and the finding that nearly half of the individuals surveyed possessed the common mtDNA haplotype. Recent studies (Wirgin et al., 1991; Turner et al., 1991) of striped bass, a species in which mtDNA restriction-site and nuclear-gene variation are extremely low, have shown considerably higher levels of variation in noncoding nuclear DNA sequences, some of which have permitted identification of discrete subpopulations or stocks. A similar situation may exist in red snapper, although this appears unlikely given the number of different (low frequency) mtDNA haplotypes observed. Third, observed homogeneity of mtDNA haplotype frequencies may reflect historical rather than present-day gene flow (i.e., red snapper could be isolated spatially in the present-day Gulf but have had sufficient genetic contact in the recent past to remain indistinguishable in mtDNA haplotype frequencies). This possibility is difficult (if not impossible) to test. Finally, low levels of gene flow could be occurring among almost completely isolated spatial subpopulations of red snapper such that frequencies of mtDNA haplotypes remain similar. This last possibility assumes equilibrium conditions and selective neutrality of different mtDNA haplotypes, both of which are difficult to test empirically.

The significant heterogeneity in levels of mtDNA variation among geographic samples of red snapper suggests localized or regional differences in effective female population size. Avise and colleagues (1988) developed methods to estimate effective female population size ($N_{f(e)}$ values) from estimates of intrapopulational (mtDNA) nucleotide sequence diversities. In theory, $N_{f(e)}$ values reflect the number of

females that gave rise to the mtDNA molecules observed. Recently, Bowen and Avise (1990) and Avise (1992) referred to $N_{f(e)}$ values as "long-term" or "evolutionary" effective female population size and suggested that differences in $N_{f(e)}$ values may signal historic, rather than present-day, variation. When applied to red snapper (and using a generation interval of 5 years), $N_{f(e)}$ values for the 3 localities examined in this study ranged from 14,000 at Port Aransas, TX, to 31,000 at both Port Fourchon, LA, and Pensacola, FL. Large or significant differences in intrapopulational mtDNA diversities (and in $N_{f(e)}$ values) among proximate geographic samples of the same marine fish species have now been reported for red snapper and Atlantic weakfish (Graves et al., 1992a). The fact that spatial differences exist within contemporary populations suggests that intrapopulational mtDNA diversities may not exclusively reflect historic events, and moreover, that such estimates may be useful in assessing reproductive capacity and condition of populations or stocks by geographic region. This hypothesis is based in part on the concept that estimates of mtDNA variability reflect genome-wide variability and that the latter affects probabilities of population survival and fitness (Soulé, 1980; Frankel and Soulé, 1981).

Levels of mtDNA variation in red snapper are low relative to other marine fishes studied to date. Not coincidentally, most of the marine fishes studied to date are of importance to commercial or recreational fishing interests, and it is worth noting that 3 species (i.e., red snapper, weakfish, and red grouper) with low levels of mtDNA variation are currently viewed as severely overfished (GMFMC, 1989, 1991; Goodyear and Phares, 1990; Vaughan et al., 1991). This association indicates that estimates of mtDNA variation may also be useful as predictors of population or stock condition among species.

Materials and Methods

Red snapper were procured by angling and from fishermen during the fall of 1990 and the spring of 1991. Specimens were obtained off-shore from Pensacola, FL (n = 25), Port Fourchon, LA (n = 36), and Port Aransas, TX (n = 25). Heart and muscle tissues were removed from each specimen and stored in liquid nitrogen for transport to Texas A&M University, where they were stored at −80°C.

Details of mtDNA extraction, precipitation, and storage may be found in Gold and Richardson (1991). Thirteen restriction endonucleases were used to digest mtDNA molecules according to manufacturer's specifications: *Apa*I, *Bcl*I, *Dra*I, *Hin*dIII, *Hpa*I, *Nco*I, *Nhe*I, *Pvu*II, *Sca*I, *Sma*I, *Sst*I, *Stu*I, and *Xba*I. Methods of agarose gel electrophoresis, transfer to nylon membranes, hybridization, and autoradiography may be found in Gold and Richardson (1991). Hybridization employed a red snapper mtDNA probe labeled with (^{32}P) dATP and (^{32}P) dCTP (New England Nuclear, sp. act.=3000 Ci/mmol/L) by random priming (Feinberg and Volgelstein, 1984). The mtDNA probe used was the entire red snapper mtDNA molecule cloned into bacteriophage lambda using Lambda DASH II arms and Gigapack II Gold Packaging extracts (Stratagene).

Red snapper mtDNA fragments (Appendix Table 1) were sized by fitting migration distances to a least-squares regression line of lambda DNA-*Hin*dIII fragment migration distances. Homology of red snapper fragment patterns from single digestions was tested by multiple, side-by-side comparisons of all variant patterns produced by each enzyme. The number of restriction sites surveyed per restriction enzyme was: *Apa*I (3), *Bcl*I (5), *Dra*I (9), *Hin*dIII (4), *Hpa*I (3), *Nco*I (4), *Nhe*I (9), *Pvu*II (3), *Sca*I (7), *Sma*I (4), *Sst*I (4), *Stu*I (10), and *Xba*I (5). All 70 restriction sites were mapped using single and double digestions. The restriction-enzyme map may be obtained upon request from the last author.

Heterogeneity of mtDNA haplotype frequencies among geographic samples was tested using (1) the G statistic (Sokal and Rohlf, 1969), (2) a Monte Carlo randomization (bootstrap) procedure (Roff and Bentzen, 1989), and (3) the *V* statistic on arcsin, square-root transformed haplotype frequencies (DeSalle et al., 1987). Nucleon diversities were calculated after Nei and Tajima (1981) and were based on the total number of mtDNA haplotypes identified by differences in restriction-enzyme fragment patterns. Intrapopulational nucleotide sequence diversities were estimated after Nei and Tajima (1981) using restriction sites (Nei and Li, 1979).

A restriction-site presence/absence matrix for individual mtDNA haplotypes was used to estimate nucleotide sequence divergence (*p*) values among haplotypes after Nei and Tajima (1981) and Nei and Miller (1990). The resulting distance values were clustered using the UPGMA algorithm (Sneath and Sokal, 1973) and a program that computes standard errors for each node in the phenogram (Nei et al., 1985). Maximum parsimony analysis of the restriction-site presence/absence employed the MULPARS and CONTREE options in version 3.0 of the Phylogenetic Analysis Using Parsimony (PAUP)

Appendix Table 1. Digestion patterns of red snapper mtDNA produced by 13 restriction enzymes. Fragment sizes are in base pairs.

ApaI	
A	B
15,800	8,800
1,000	7,000
	1,000

BclI				
A	B	C	D	E
12,300	10,900	12,300	15,400	8,300
3,100	3,100	4,500	1,400	4,000
1,400	1,400[a]			3,100
				1,400

HindIII	
A	B
8,100	9,700
4,100	4,100
3,000	3,000
1,600	

DraI				
A	B	C	D	E
9,000	9,000	9,000	6,500	9,000
2,800	3,200	2,400	2,800	2,600
2,400	2,400	2,000	2,500	2,400
1,200	1,200	1,200	2,400	1,200
1,000	1,000	1,000	1,200	1,000
(400)[b]		800	1,000	(600)
		(400)	(400)	

HpaI	
A	B
12,000	6,000[a]
4,800	4,800

PvuII	
A	B
12,100	16,800
3,900	
800	

NcoI		
A	B	C
14,800	10,600	14,800
2,000	4,200	1,400
	2,000	(600)

ScaI	
A	B
6,000[a]	6,000[a]
2,200	2,200
1,200	1,800
(600)[a]	(600)
(200)	(200)

SstI	
A	B
10,800	10,800
3,300	2,700
2,700	2,100
	1,200

XbaI	
A	B
7,200	7,200
4,800	3,600
3,000	3,000
1,800	1,800
	1,200

NheI				
A	B	C	D	E
9,100	9,100	9,100	4,700[a]	5,200
4,700	4,700	3,900	4,400	4,700
1,300	1,200	1,300	1,300	3,900
1,200	1,000	1,200	1,200	1,300
(500)	(500)	800	(500)	1,200
	(300)	(500)		(500)

StuI		
A	B	C
5,600	4,300	5,600
4,100	4,100	2,700
2,700	2,700	2,400
1,700	1,700	1,700[a]
1,200	1,300	1,200
700	1,200	700
600	700	600
(200)	(600)	(200)
	(200)	

SmaI	
A	B
8,600	8,600
3,700	7,200
3,500	1,000
1,000	

[a]Fragment "doublets" determined from mapping.

[b]Parentheses indicate fragments not normally seen in autoradiographs but known to exist from mapping.

program of Swofford (1991). All autapomorphic and symplesiomorphic characters were removed prior to analysis using PAUP. Minimum-length parsimony networks of mtDNA haplotypes were constructed by connecting composite haplotypes in increments of single gains or losses. Organization of mtDNA data for analysis was facilitated by the Restriction Enzyme Analysis Package (REAP) of McElroy et al. (1992).

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