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Mitochondrial DNA variation in red grouper (*Epinephelus morio*) and greater amberjack (*Seriola dumerili*) from the Gulf of Mexico

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The purposes of this study were to: (i) develop mitochondrial (mt)DNA probes for two species-groups of reef fishes, and (ii) assay mtDNA restriction fragment length polymorphism (RFLP) variation in two reef fish species from the Gulf of Mexico. MtDNA probes for groupers of the genera *Epinephelus* and *Mycteroperca* and for jacks of the family Carangidae were generated and mtDNA fragment patterns of 34 enzymes were assayed from samples of red grouper (*Epinephelus morio*) and greater amberjack (*Seriola dumerili*). Ten polymorphic restriction enzymes (yielding 12 distinct mtDNA haplotypes) were identified in the red grouper, while 19 polymorphic enzymes (yielding 23 distinct mtDNA haplotypes) were identified in greater amberjack. Estimates of percentage nucleotide-sequence divergence among haplotypes ranged from 0.09–0.59 (mean ± S.E. = 0.29 ± 0.02) in red grouper and from 0.09–1.32 (mean ± S.E. = 0.49 ± 0.02) in greater amberjack. Estimated mtDNA nucleon diversity values were 0.418 and 0.900 for red grouper and greater amberjack, respectively. Estimated intrapopulational percentage nucleotide diversities were 0.078 ± 0.003 (mean ± S.E.) for red grouper and 0.336 ± 0.006 (mean ± S.E.) for greater amberjack. Estimates of the evolutionary effective female population size ($N_{f(e)}$) were generated for both species and compared to $N_{f(e)}$ values for eight other marine fish species or subspecies. The $N_{f(e)}$ value for greater amberjack was more or less intermediate, whereas the $N_{f(e)}$ value for red grouper was low. The latter may indicate that red grouper warrant immediate management attention. Heterologous hybridizations to mtDNAs of two other species of *Epinephelus* (*E. adscensionis* and *E. fulvus*) and to *Mycteroperca phenax* (using the red grouper probe), and to mtDNA of the horse-eye jack, *Caranx latus* (using the greater amberjack probe), were successful. MtDNA data generated from red grouper and greater amberjack, as well as the potential to obtain mtDNA data from other grouper and carangid species, should prove useful in future studies of genetic variation and population differentiation in these important reef fishes.

Key words: mitochondrial DNA, reef fish, red grouper, greater amberjack, Gulf of Mexico.

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Introduction

Reef fishes represent an important recreational and commercial resource in the Gulf of Mexico. Recently, concern has been expressed over the future of several reef fish species. Reasons for the concern are: (i) declining commercial and recreational landings of many reef fish species, and (ii) estimates of spawning stock biomass which, in several species, have fallen to less than minimum desired values (GMFMC, 1989, 1990; Goodyear, 1988; Goodyear and Phares, 1990). Detailed information on biological characteristics and abundance are available for only a few reef fish species (Goodyear and Phares, 1990; Bullock and Smith, 1991), and almost no information is available on genetic stock structure or levels of genetic variability in any reef fish species from the Gulf of Mexico. The need for information on genetic stock structure of Gulf reef fishes is critical in terms of possible identification of genetically meaningful management units. An additional reason for needing information on genetic stock structure, particularly in reef fish species, regards the positive identification of larval or juvenile individuals with respect to stock or even species of origin. Finally, information on levels of relative genetic variability within species may be critical in terms of identifying populations or species in which reductions in effective population size

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may have occurred. The concept that levels of genetic variability may affect probabilities of survival and populational fitness has been discussed by Soulé (1980) and Frankel and Soulé (1981), and several authors (Vrijenhoek and Lerman, 1982; Quattro and Vrijenhoek, 1989; Wildt *et al.*, 1987) have used genetic characters to assess levels of genome-wide variability.

The central purpose of this study was to generate a foundation for genetic assessment of stocks in two key reef fish groups, viz. groupers of the serranid genera *Epinephelus* and *Mycteroperca* and jacks of the family Carangidae. Several grouper or jack species are, or soon will be, incorporated into the Fishery Management Plan for the Reef Fish Resources of the Gulf of Mexico and subjected to adjusted management measures (GMFMC, 1989, 1990). Objectives of the present work were: (i) to construct mitochondrial (mt)DNA probes that would hybridize to mtDNAs of both groupers and jacks, and (ii) to generate data on mtDNA variation in an example species from each group. The two species chosen for study were red grouper (*Epinephelus morio*) and greater amberjack (*Seriola dumerili*). Both species are important components of the recreational and commercial fishery in the Gulf and both are currently "restricted" in terms of management regulations. Red grouper occur exclusively in the Atlantic and are found from the coast of Massachusetts southward to Brazil (Smith, 1961). Greater amberjack are found in the Mediterranean Sea and the Atlantic, Pacific, and Indian Oceans; in the Atlantic, they occur from Nova Scotia to Brazil (Manooch, 1988). Adults of both species are typically concentrated around reef or reef-like structures, including ledges, crevices, and caverns of rocky limestone reefs, rocky outcroppings, or ship wrecks (Manooch, 1988).

The genetic tool utilized in the study was restriction fragment length polymorphism (RFLP) analysis of mitochondrial (mt)DNA. Recent stdies in a variety of organisms, including fishes, have shown that RFLP analysis of mtDNA is more powerful than protein electrophoresis in differentiating stocks or subpopulations within species (Bernatchez and Dodson, 1991; Avise, 1986, 1987; Avise *et al.*, 1987; Moritz *et al.*, 1987). Briefly, mtDNA is a genetically haploid, physically circular molecule which is uniclonally inherited through the maternal parent. This means that mtDNA sequence variants do not segregate and recombine during sexual reproduction, and that mtDNA molecules within single individuals should be essentially identical in size and sequence. The joint effects of haploidy and maternal inheritance means that mtDNA should be four times more sensitive (compared to nuclear-encoded genes) in assessing the genetic impact of population subdivision (Birky *et al.*, 1983; Templeton, 1987). Finally, mtDNA appears to have a rapid rate of sequence evolution, which means that mtDNA analysis should be useful in identifying discrete stocks or breeding subpopulations of recent origin (Avise, 1987).

Materials and methods

Sampling

Individuals of both species were procured either by angling or from fishermen during the fall of 1990 and the spring of 1991. Red grouper were obtained from the Dry Tortugas, Florida (n = 5), and from the Middle Grounds off the west coast of Florida near Sarasota (n = 46). Greater amberjack were obtained offshore from Sarasota, Florida (n = 32), and Pensacola Beach, Florida (n = 24). Single individuals of greater amberjack were also obtained from the Dry Tortugas, Florida, and offshore from Port Fourchon, Louisiana, and Port Aransas, Texas. Individuals of other grouper or carangid species, i.e. coney (*Epinephelus fulvus*), rock hind (*Epinephelus adscensionis*), scamp (*Mycteroperca phenax*), and horse-eye jack (*Caranx latus*) were procured from the Dry Tortugas, Florida. Heart and muscle tissues were removed from all individuals and stored in liquid nitrogen for transport to our laboratory in College Station, Texas. Tissue samples were stored at −80°C in an ultracold freezer.

Restriction enzyme survey

The assay of mtDNAs of individual fish and details of DNA extraction, precipitation, and storage may be found in Gold and Richardson (1991). Thirty-four six-base restriction endonucleases were used to digest 1.0–1.5 µg of DNA in 40 µl reactions following the manufacturer's specifications. Enzymes used were: *Alw*44I, *Apa*I, *Bam*HI, *Bcl*I, *Bgl*II, *Bst*EII, *Cla*I, *Csp*45I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Kpn*I, *Mlu*I, *Nar*I, *Nco*I, *Nde*I, *Nhe*I, *Nru*I, *Nsi*I, *Pst*I, *Pva*I, *Pvu*II, *Sal*I, *Sca*I, *Sma*I, *Spe*I, *Ssp*I, *Sst*I, *Sst*II, *Stu*I, *Xba*I, *Xho*I, and *Xmn*I. Methods of agarose electrophoresis, transfer to nylon membranes, hybridization, and autoradiography may be found in Gold and Richardson (1991). Hybridization employed mtDNA probes (see below) labelled with (^{32}P)dCTP (New England Nuclear, sp. act. = 3000 Ci/mM) by random priming (Feinberg and Volgenstein, 1984). After autoradiography, mtDNA fragments were sized by fitting migration distances to a least-squares regression line of lambda DNA-*Hin*dIII fragment migration distances.

Cloning

Purified genomic DNA from both species was digested to completion with *Bam*HI. Following digestion, DNA fragments were electrophoresed in 0.8% agarose gels, transferred to nylon filters, and hybridized to a redfish (*Sciaenops ocellatus*) mtDNA probe (Gold and Richardson,

1991) at low stringency. Autoradiography revealed a single *Bam*HI site in red grouper mtDNA and up to three *Bam*HI sites in greater amberjack mtDNA. Approximately 1 μg of purified genomic DNA from red grouper was then digested to completion with *Bam*HI, while a similar quantity of greater amberjack DNA was partially digested. The DNAs were then ligated into *Bam*HI-digested Lambda DASH II arms and packaged using Gigapack II Gold Packaging extracts (Stratagene). The resulting genomic libraries of approximately 5×10^4 recombinant phages each were then screened for mtDNA inserts using polymerase chain reaction (PCR) amplified mtDNA fragments from the cyprinid fish *Cyprinella lutrensis*. A single recombinant phage containing the entire red grouper mtDNA molecule was isolated and designated Em-mt2; a single recombinant phage containing ca 12.5 kb of the greater amberjack mtDNA genome was also isolated and was designated Sd-mt2A.

Data analyses

Genotypic ("nucleon") diversity values within geographic samples and among all individuals examined were calculated for each species following 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 diversity was estimated using equations in Nei and Tajima (1981). Heterogeneity of mtDNA haplotype frequencies between the two samples of greater amberjack from Florida was tested using the V statistic on arcsin square-root transformed haplotype frequency values (after DeSalle *et al.*, 1987) and the chi-square bootstrap analysis described in Roff and Bentzen (1989). The latter was carried out using the MONTE program in the restriction enzyme analysis package (REAP) of McElroy *et al.* (1992).

Restriction site presence/absence matrices for individual mtDNA haplotypes in each species were constructed using the GENERATE program in REAP by inferring restriction site gains or losses for each enzyme. The DSE program in REAP was used to estimate nucleotide sequence divergence (p) values (Nei and Tajima, 1981; Nei and Miller, 1990) among haplotypes. The resultant distance matrices were clustered using the UPGMA algorithm (Sneath and Sokal, 1973) and a computer program written by Dr N. Saitou of the University of Texas Health Science Center in Houston. This program also computes a standard error for each node in the phenogram according to equations in Nei *et al.* (1985).

Minimum-length parsimony networks of mtDNA haplotypes were constructed for each species by connecting composite haplotypes in increments of single site gains or losses. Maximum parsimony analysis, employing the MULPARS and CONTREE options in version 3.0 of the phylogenetic analysis using parsimony (PAUP) program of Swofford (1991), was carried out on the restriction site presence/absence matrix of greater amberjack. All autapomorphic and symplesiomorphic characters were removed prior to analysis using the REDUCE program in REAP. This analysis was not carried out for red grouper since 89 of the 92 restriction site characters in the red grouper presence/absence matrix were either autapomorphic or symplesiomorphic.

Other species

In order to examine the applicability of the mtDNA probes on other grouper or jack species, heterologous hybridizations were carried out using genomic DNAs isolated from coney (*E. fulvus*), rock hind (*E. adscensionis*), scamp (*Mycteroperca phenax*), and horse-eye jack (*C. latus*) and the procedures outlined above. Hybridization conditions, however, were relaxed slightly by reducing the temperature of hybridization to 60°C and increasing the amount of probe DNA per genomic blot.

Results and discussion

Red grouper

Single digestions of mtDNA molecules from the 51 red grouper surveyed using 34 restriction enzymes produced a total of 92 inferred, unique restriction sites. Six of the enzymes (*Bst*EII, *Cla*I, *Nar*I, *Nru*I, *Pvu*I, and *Sal*I) did not digest red grouper mtDNA, while another seven (*Alw*44I, *Bam*HI, *Csp*45I, *Eco*RV, *Mlu*I, *Sma*I, and *Sst*I) recognized only a single restriction site. Single digestions with the remaining 21 restriction enzymes produced at least two mtDNA fragments of varying sizes (Appendix Table A1). The mean genome size of all apparently complete digestion patterns was 16.8 ± 0.2 kb. No evidence for mtDNA size variation or heteroplasmy was observed among the individuals surveyed.

Ten of the restriction enzymes surveyed produced variant digestion patterns, all of which were consistent with the hypothesis of single nucleotide substitutions (i.e. single gains or losses of restriction sites) among variant patterns for each enzyme. Digestion patterns of the 10 "polymorphic" restriction enzymes produced a total of 12 composite mtDNA haplotypes among the 51 individuals screened (Table 1). Of the 12 haplotypes, haplotype 1 accounted for greater than 76% of the individuals surveyed, and 10 of the remaining haplotypes were found in only one individual each. Haplotype 1 was found in all five individuals sampled from the Dry Tortugas. Estimates of the percentage nucleotide sequence divergence

Table 1. Distribution of 12 mitochondrial DNA composite digestion patterns (haplotypes) in two geographic samples of red grouper.

Haplotype no.	Composite MtDNA genotype*	Locality†	
		DRT	SAR
1	AAAAAAAAAA	5	34
2	AAABAAAAAA	–	1
3	AAAAAAAAAB	–	2
4	AAAABAAAAA	–	1
5	ABAAAABAAC	–	1
6	AAAAAABAAA	–	1
7	AABAAABAAA	–	1
8	ABAAAABCAB	–	1
9	CAAAAAAAAA	–	1
10	AAAAABAAAA	–	1
11	AAAAAAABBA	–	1
12	BAAAAAAAAB	–	1

*Letters (from left to right) are digestion patterns (Appendix Table A1) for *Apa*I, *Kpn*I, *Nco*I, *Nde*I, *Nhe*I, *Nsi*I, *Pvu*II, *Ssp*I, *Xba*I, and *Xmn*I.
†Locality abbreviations are DRT (Dry Tortugas, Florida) and SAR (Sarasota, Florida).

among the 12 haplotypes ranged from 0.09 to 0.59 (mean ± S.E. = 0.29 ± 0.02).

MtDNA nucleon diversity, estimated using all 51 individuals, was 0.418. This value represents an index of mtDNA polymorphism (Avise *et al.*, 1989) which ranges from zero (all individuals have the same mtDNA haplotype) to one (all individuals have different mtDNA haplotypes). Intrapopulational nucleotide sequence diversity, estimated for all 51 individuals, was 0.078 ± 0.003 (mean ± S.E.) %.

Phenetic relationships (using UPGMA clustering of genetic distances) and a parsimony network of the 12 red grouper haplotypes are shown in Figure 1. In the UPGMA phenogram (Fig. 1a), all 12 haplotypes were joined at 0.44% nucleotide sequence divergence. Haplotypes 5 and 8 were the most divergent, although the difference between these two haplotypes and the remainder is non-significant. The parsimony network (Fig. 1b) includes a single, "assumed" haplotype (i.e. a haplotype not detected in the survey). Including the "assumed" haplotype, all of the haplotypes can be derived from one another by one or two mutational steps. Haplotype 1 was considered central because it was the most common haplotype observed and because all but haplotypes 5 and 8 can be derived from haplotype 1 by one or two steps. The phenetic and parsimony analyses indicate a high degree of genetic similarity among the 12 haplotypes, suggesting that all 12 are recently evolved. If divergence times are estimated based on the "conventional" rate of evolution for vertebrate mtDNA of 0.01 substitutions/bp/lineage/Myear (Brown *et al.*, 1979; Wilson *et al.*, 1985), the average per cent nucleotide sequence divergence among the 12 haplotypes (0.29) suggests an average divergence time among the haplotypes of approximately 150 000 years. The two most divergent red grouper haplotypes (5 and 11) differed by 0.59%, suggesting a maximum divergence time of 295 000 years.

Greater amberjack

Single digestions of mtDNA molecules from the 59 greater amberjack surveyed using 34 restriction enzymes produced a total of 102 inferred, unique restriction sites. Two of the enzymes (*Kpn*I and *Nar*I) did not digest greater amberjack mtDNA, while another three (*Bst*EII, *Nru*I, and *Xho*I) recognized only a single mtDNA restriction site. Single digestions with the remaining 29 restriction enzymes produced at least two mtDNA fragments of varying sizes (Appendix Table A2). Note that not all digestion patterns produced fragments whose sizes summed to 16.9 kb, the inferred genome size of the greater amberjack mtDNA. The reason for the latter is that the Sd-mt2A probe does not cover the entire greater amberjack mtDNA molecule, i.e. fragments in some digestion patterns are undetected. The mean genome size of all apparently complete digestion patterns was 16.9 ± 0.2 kb. No evidence for mtDNA size variation or heteroplasmy was observed among the individuals surveyed.

Nineteen of the restriction enzymes produced variant digestion patterns, all of which were consistent with the hypothesis of single nucleotide substitutions among variant patterns for each enzyme. Digestion patterns of the 19 "polymorphic" restriction enzymes produced a total of 23 composite mtDNA haplotypes among the 59 individuals screened (Table 2). Two haplotypes (1 and 4) were the most abundant, each occurring in 12 individuals. Haplotypes 3, 5, and 6 occurred in four or five individuals and 15 of the haplotypes were found in only one individual each. Of the haplotypes found in more than one individual, only haplotype 6 was unique to a single locality (SAR). Estimates of the percentage nucleotide sequence divergence among the 23 haplotypes ranged from 0.09 to 1.32 (mean ± S.E. = 0.49 ± 0.02). MtDNA nucleon diversity was 0.900, and intrapopulational nucleotide sequence diversity was 0.336 ± 0.006 (mean ± S.E.) %. These estimates were based on all 59 individuals surveyed, since both V tests and chi-square bootstrap analysis of geographic heterogeneity in mtDNA haplotype frequencies were non-significant.

Phenetic relationships (from UPGMA clustering) and a parsimony network of the 23 greater amberjack haplotypes are shown in Figure 2. The UPGMA phenogram (Fig. 2a) revealed three groupings: one consisting only of haplotype 22, one consisting of six haplotypes (3, 6, 9, 11, 15, 20), and one consisting of the remaining 16 haplotypes. Based on the standard errors, the differences

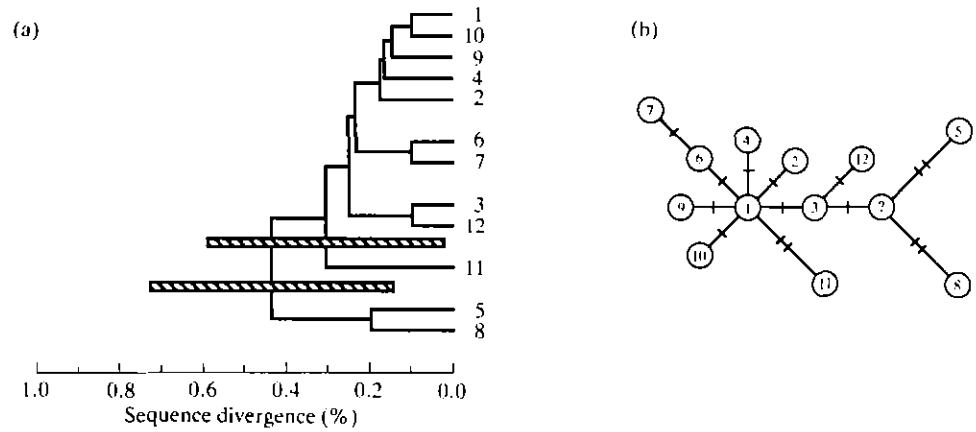

Figure 1. (a) UPGMA cluster analysis of percentage nucleotide sequence divergence values. Operational units are the 12 composite mtDNA genotypes (haplotypes) found in red grouper. Hatched bars are standard errors of the nodes which they overlie. (b) Parsimony network of 12 haplotypes found in red grouper. 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.

Table 2. Distribution of 23 mitochondrial DNA composite digestion patterns (haplotypes) among geographic samples of greater amberjack.

Haplotype no.	Composite MtDNA genotype*	Locality†				
		DRT	SAR	PSB	PTF	PAR
1	AAAAAAAAAAAAAAAAAAA	1	6	5	–	–
2	AAAAABBAAAAAAAAAAAA	–	–	1	1	–
3	AAAAAABAAAAAAAAAABA	–	–	3	–	1
4	AAAAAABAAAAAAAAAAAA	–	9	3	–	–
5	AAABBABAAAAAABAABAA	–	1	3	–	–
6	AAAAAAAAAAAAAAAAABA	–	5	–	–	–
7	AAAAAAAAAAAAAABAAAA	–	1	–	–	–
8	AAAAAABAAABAAAAAACA	–	1	–	–	–
9	BAABAAAAAAAAACAAABA	–	1	–	–	–
10	BAAAAABBABAAAAAAAAA	–	1	–	–	–
11	BAABAAAAAAAAAAAAABA	–	1	–	–	–
12	AAAAAAAAAAAABACAAAA	–	1	–	–	–
13	AAAAAAAAAAAABABAAAA	–	2	1	–	–
14	AAAAAAAABAAAAAAAAAA	–	1	–	–	–
15	AAAAAAAAAAAAAAAABABA	–	1	–	–	–
16	AAAAAABAAABAAAAAAAA	–	1	1	–	–
17	AABAAABAAAABAAAAAAC	–	–	1	–	–
18	AABAAABAAAAAAAAAAAA	–	–	1	–	–
19	AAAAAABAACAAAAAAAAA	–	–	1	–	–
20	AAAAAAAAAAAAAAAAABB	–	–	1	–	–
21	AAAAAABAABAAAAAAAAA	–	–	1	–	–
22	ABAAACAAAAACBAAAABA	–	–	1	–	–
23	AAABAABAAAAAAAAAAAA	–	–	1	–	–

*Letters (from left to right) are digestion patterns (Appendix Table A2) for *Afl*III, *Apa*I, *Cla*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Nco*I, *Nsi*I, *Pst*I, *Pvu*I, *Pvu*II, *Sca*I, *Sma*I, *Spe*I, *Ssp*I, *Sst*I, *Stu*I, and *Xba*I.
†Locality abbreviations are DRT (Dry Tortugas, Florida), SAR (Sarasota, Florida), PSB (Pensacola Beach, Florida), PTF (Port Fourchon, Louisiana), and PAR (Port Aransas, Texas).

among haplotypes in the two larger groupings do not appear significant. There appears to be little geographic cohesion to either of the two larger groupings, although four of the haplotypes (6, 9, 11, 15) in the smaller of the two groupings were found only at the SAR locality (Table 2). The parsimony network (Fig. 2b) required a minimum

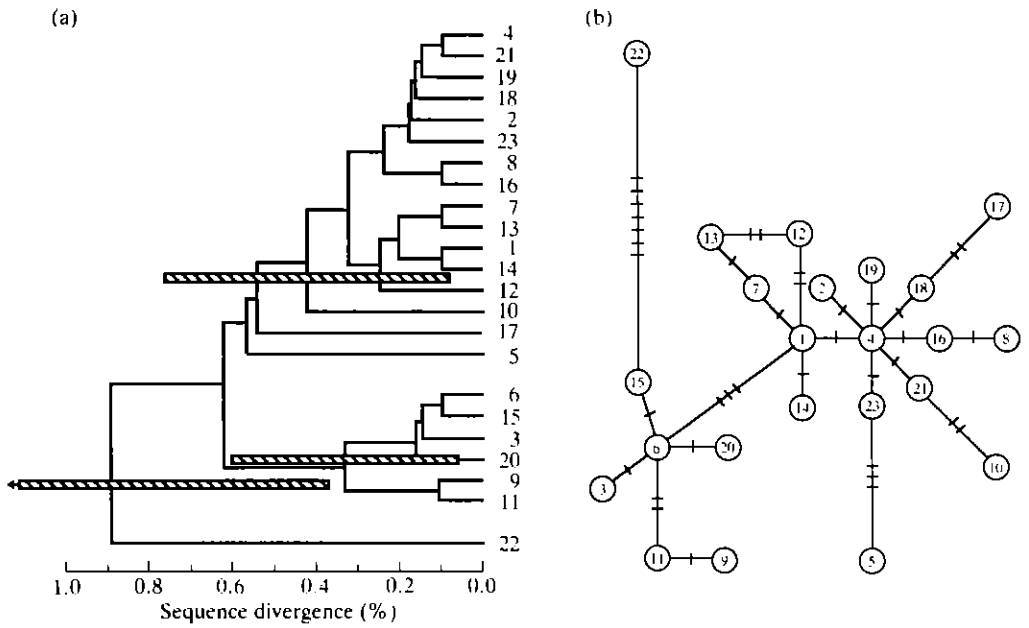

Figure 2. (a) UPGMA cluster analysis of percentage nucleotide sequence divergence values. Operational units are the 23 composite mtDNA genotypes (haplotypes) found in greater amberjack. Hatched bars are standard errors of the nodes which they overlie. (b) Parsimony network of 23 haplotypes found in greater amberjack. Branches connecting haplotypes are drawn proportional to the number of restriction site changes (hatch marks) required to connect adjacent haplotypes.

Table 3. Comparisons of estimates of mtDNA variability and of evolutionary effective population sizes for females among species of marine fishes.

	Number of individuals surveyed	Number of different mtDNA haplotypes	Nucleon diversity	Nucleotide sequence diversity (%)	Evolutionary effective female population size $[N_{f(e)}]$*
Atlantic menhaden (Avise, 1992)	17	17 (55)†	1.00	3.19	800 000
Atlantic herring (Kornfield and Bogdanowicz, 1987)	69	26 (77)	0.93	1.09	273 000
Gulf menhaden (Avise, 1992)	16	16 (55)	1.00	0.99	250 000
Red drum (Gold and Richardson, 1993)	887	116 (100)	0.95	0.57	95 000
Spotted seatrout (Furman and Gold, pers. comm.)	70	14 (50)	0.53	0.30	75 000
Greater amberjack (this paper)	59	23 (102)	0.90	0.34	57 000
Black drum (Furman and Gold, pers. comm.)	250	19 (67)	0.60	0.17	17 000
Red grouper (this paper)	51	12 (92)	0.42	0.08	10 000
Black sea bass‡ (Avise, 1992)	19	3 (61)	0.21	0.03	5000
Black sea bass§	10	2 (61)	0.22	0.03	5000

*Assumed generation lengths for each species are given in text.
†Parentheses indicate number of restriction sites or fragments surveyed.
‡Atlantic subspecies (*Centropristis striata striata*).
§Gulf subspecies (*Centropristis striata melana*).

36 steps, assuming that haplotype 12 is derived from either haplotype 1 or haplotype 13, but not both. The "triangle" shown for these three haplotypes in Figure 2b reflects that haplotype 12 could be derived from either haplotype 1 or haplotype 13 by two steps. Maximum parsimony analysis (using PAUP) of the 23 haplotypes

generated multiple, equally parsimonious "trees". The strict consensus tree produced using the CONTREE option of PAUP (data not shown) yielded a single clade comprised of 3, 6, 9, 11, 15, 20, and 22, i.e. the smaller of the two groupings (plus haplotype 22) shown in Figure 2a. The remaining 16 haplotypes were represented as single lineages forming a large basal polytomy along with the clade comprised of the above seven haplotypes.

The phenetic and parsimony analyses indicated a higher degree of genetic dissimilarity among greater amberjack haplotypes than was found among red grouper haplotypes. The average per cent nucleotide sequence divergence among the 23 greater amberjack haplotypes was 0.49, suggesting an average divergence time among the haplotypes of approximately 245 000 years. The two most divergent greater amberjack haplotypes (5 and 22) differed by 1.32%, suggesting a maximum divergence time of 660 000 years.

Comparisons

The estimates of mtDNA intrapopulational nucleotide sequence diversity in both red grouper and greater amberjack were used to generate estimates of evolutionary effective female population sizes ($N_{f(e)}$) using methods outlined in Avise *et al.* (1988). The estimates of $N_{f(e)}$ and of other parameters for both species are shown in Table 3, along with comparable estimates for eight other marine fish species or subspecies of commercial or recreational importance. Data for the latter were taken either from the published literature or from work in our laboratory. Assumed generation lengths for the taxa were 2 years (Atlantic and Gulf menhaden, Atlantic herring, and spotted seatrout), 3 years (red drum, greater amberjack, and black sea bass), and 5 years (black drum and red grouper), and were based either on Avise (1992) or personal communications from L. Bullock (red grouper), I. Kornfield (Atlantic herring), and C. Wilson (spotted seatrout, red drum, greater amberjack, black drum). As shown in Table 3, estimates of $N_{f(e)}$ are highest for the clupeid species (menhaden and herring), intermediate for red drum and spotted seatrout (two sciaenids) and greater amberjack, and lowest for black drum (a sciaenid), red grouper, and black sea bass (both serranids).

The data in Table 3 indicate that levels of extant genetic variation vary considerably among the species assayed. With respect to the two species examined in this study, the $N_{f(e)}$ value of 57 000 for the greater amberjack indicates moderate levels of mtDNA genetic variability in comparison to other, non-clupeid species and higher levels than found in the other two reef fish species (viz. the red grouper and black sea bass). Minimally, the estimated $N_{f(e)}$ value for greater amberjack provides a baseline figure against which future estimates can be compared. This may prove important given the steady increase in commercial and recreational landings of this species in the Gulf of Mexico over the past few years (GMFMC, 1989). The low $N_{f(e)}$ value of 10 000 observed in red grouper indicates reduced mtDNA genetic variability in this species and the possibility that red grouper may warrant immediate attention in terms of effective management regulation. Commercial and recreational landings of red grouper in the Gulf of Mexico have steadily decreased in the past few years (GMFMC, 1989), suggesting that population sizes may also have decreased. One final point to note is that the two species with the lowest estimated $N_{f(e)}$ values (i.e. the red grouper and black sea bass) are both protogynous hermaphrodites, i.e. individuals are first females and then transform to males (Manooch, 1988). This alternative life-style may possibly affect estimates of $N_{f(e)}$, although the extent of such an effect (if it occurs) is unknown.

Other species

Hybridizations of the greater amberjack probe to mtDNA of horse-eye jack (*C. latus*) were successful, as were hybridizations of the red grouper probe to mtDNAs of *E. fulvus*, *E. adscensionis*, and *M. phenax*. This demonstrated that the mtDNA probes generated in this study may be used in studies of mtDNA variation in grouper and carangid species.

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Appendix

Table A1. Digestion patterns (fragment sizes in base pairs) of red grouper mtDNA produced by 21 six-base restriction endonucleases. Parentheses indicate fragments not seen but assumed to exist. Asterisks denote variable or "polymorphic" restriction enzymes.

*Bcl*I	*Eco*RI	*Kpn*I*		*Nco*I*		*Nde*I*		*Nsi*I*		*Sst*II	*Xho*I
A	A	A	B	A	B	A	B	A	B	A	A
8700	12 600	15 250	7625	13 800	16 800	12 900	15 200	16 800	9100	14 850	10 600
8100	4200	1550	7625	3000		2300	1600		7700	1700	6200
			1550							(250)	

	*Apa*I*		*Bgl*II	*Hin*dIII	*Hpa*I	*Pst*I	*Pvu*II*		*Xba*I*	
A	B	C	A	A	A	A	A	B	A	B
10 300	15 400	10 300	9450	10 600	8000	10 700	7000	9550	9200	9200
5100	1400	5100	5800	2300	5100	3600	6200	6200	3300	4300
1400		1150	900	2100	3100	1500	2550	1050	2300	3300
		(250)	650	1800	(600)	1000	1050		2000	

*Nhe*I*		*Sca*I	*Spe*I		*Ssp*I*		*Stu*I		*Xmn*I*	
A	B	A	A	A	B	C	A	A	B	C
6800	6800	6800	4150	6000	6000	6000	4800	6400	8100	8100
3300	3300	4200	3650	4800	3400	4800	4400	3600	3600	5550
2950	2950	3500	3400	2900	2900	2900	4100	1950	1950	1700
2450	2100	1800	2800	1700	1700	1450	1600	1700	1700	1450
1300	1300	(500)	1100	800	1400	800	1200	1700	1450	
	(350)		1000	600	800	600	700	1450		
			700		600	(250)				

Table A2. Digestion patterns (fragment sizes in base pairs) of greater amberjack produced by 29 six-base restriction endonucleases. Parentheses indicate fragments not seen but assumed to exist. Asterisks denote variable or "polymorphic" restriction enzymes.

*Bcl*I	*Bgl*II	*Bst*EII	*Hin*dIII*			*Mlu*I	*Nsi*I*		*Pvu*I*		*Sal*I
A	A	A	A	B	C	A	A	B	A	B	A
13 800	12 400	16 200	16 900	14 300	9700	13 000	16 900	13 000	10 600	16 900	15 700
(3100)	4500	(700)		2600	7200	3900		3900	6300		1200

*Alw*44I*		*Bam*HI	*Cla*I*		*Csp*45I	*Eco*RI*		*Eco*RV*		*Nhe*I	*Sst*II
A	B	A	A	B	A	A	B	A	B	A	A
15 150	16 900	10 600	16 900	9900	7900	8700	16 900	9800	9800	13 800	14 000
1750		1900		7000	6500	8200		3900	7100	2600	1700
		(4 400)			2500			3200		500	(1200)

*Hpa*I*		*Nco*I*		*Pst*I*			*Sma*I*			*Ssp*I*	
A	B	A	B	A	B	C	A	B	C	A	B
8800	8800	11 050	8450	13 900	13 900	13 900	10 700	10 700	11 150	10 700	8200
8100	4500	4200	4200	3000	1500	2350	5750	6200	5750	6200	6200
	3600	1650	2600		(1500)	650	(450)				(2500)
			1650								

*Apa*I*		*Pvu*II*			*Sca*I*		*Spe*I*			*Sst*I*	
A	B	A	B	C	A	B	A	B	C	A	B
9500	9500	6250	13 450	6250	10 000	6900	7900	6500	11 400	8500	8500
5700	5700	4900	3000	5750	3600	3600	3500	3500	2300	3450	3450
1000	500	3000	(450)	4900	2700	3100	2300	2300	1200	3200	3200
(700)	(1200)	2300			600	2700	1200	1400	800	1750	1650
		(450)				600	800	1200	(1200)		(100)
							(1200)	800			
								(1200)			

Table A2. *Continued.*

*Stu*I*			*Xba*I*			*Xmn*I
A	B	C	A	B	C	A
6500	5500	6500	7300	9700	7300	4900
4400	4400	4100	4600	4600	4300	4700
1700	1700	1700	2600	2600	2600	4400
(4300)	1200	(4600)	2400		2400	1300
	(4100)				(300)	(1600)