Mitochondrial DNA diversity in and population structure of red grouper, *Epinephelus morio*, from the Gulf of Mexico*

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Red grouper, *Epinephelus morio*, is a protogynous hermaphrodite found exclusively in the Atlantic Ocean from the coast of Massachusetts southward to Brazil (Smith, 1961). It is most abundant along the western Florida shelf and off the north coast of the Yucatan Peninsula, Mexico (Brulé and Canché, 1993). Studies on the biology of red grouper are few. Adults are known to be associated with rocky reef bottoms and caverns, ledges, and crevices formed by limestone outcroppings (Moe, 1969). Other available data include food habits and some aspects of early life history and patterns of migration (Moe, 1969; Brulé and Canché, 1993).

Red grouper are important to both commercial and recreational fisheries in the United States (U.S.) and Mexico (Moe, 1969). In recent years, declines in recreational and commercial landings have led to regulation of both fisheries in U.S. waters. The Mexican red grouper fishery, reportedly working above maximum sustainable yield (Solís Ramírez, 1970; Arreguín-Sánchez, 1987), however, remains essentially unregulated. Important in formulating management plans for marine fish species such as red grouper is information on population structure or stocks and on levels of genetic variation. This information is critical for both stock assessment and adjustment of fishery regulations within regions.

In a previous study (Richardson and Gold, 1993), we examined mitochondrial (mt)DNA variation among a sample of red grouper from the west coast of Florida. Estimated within-population mtDNA diversity in this sample was among the lowest reported for a marine fish species. In this note, we report mtDNA variation within a sample of red grouper from the Campeche Banks, Mexico (Fig. 1). The main objectives were 1) to determine whether red grouper from Florida and Mexico represent different genetic stocks and 2) to compare levels of mtDNA diversity in red grouper from Campeche Banks, Mexico, with those from west Florida.

Materials and methods

Specimens were obtained from commercial fishermen in Celestún and San Felípe, Mexico, during November, 1991 (Fig. 1). Heart and muscle tissue were removed from each individual, stored at –20°C in a fish house in Merida, Mexico, and transported on wet ice to Houston, Texas, where they were frozen in liquid nitrogen. Upon arrival at Texas A&M, tissues were stored at –80°C.

Details of DNA isolation, storage, restriction enzyme digestion, agarose electrophoresis of DNA fragments, and Southern blot hybridization with a mtDNA probe may be found in Gold and Richardson (1991). The mtDNA probe used (λEm-mt2) was an entire red grouper mtDNA genome cloned into lambda bacteriophage (Richardson and Gold, 1993). The ten restriction endonucleases used in this study were those previously identified to be polymorphic in red grouper (Richardson and Gold, 1993) and included *Apa*I, *Kpn*I, *Nco*I, *Nde*I, *Nhe*I, *Nsi*I, *Pvu*II, *Ssp*I, *Xba*I, and *Xmn*I.

Within-sample mtDNA diversity was assessed by nucleon diversity (probability that any two individuals drawn at random will differ in mtDNA haplotype) and by intrapopulational nucleotide sequence diversity (average nucleotide difference between any two individuals drawn at random). Both estimates of mtDNA diversity were generated by using equations in Nei and Tajima (1981).

Geographic partitioning of mtDNA variation was assessed by homogeneity testing of mtDNA haplotype frequencies and by searching for phylogeographic cohesion or structure of mtDNA haplotypes with a parsimony approach. Homogeneity tests included 1) a log-likelihood (G) test and 2) a Monte Carlo randomization procedure developed by Roff and Bentzen (1989). Analyses were carried out with the BIOM-PC (a package of statistical programs, Applied Biostatistics Inc.; Rohlf, 1987) and REAP (restriction en-

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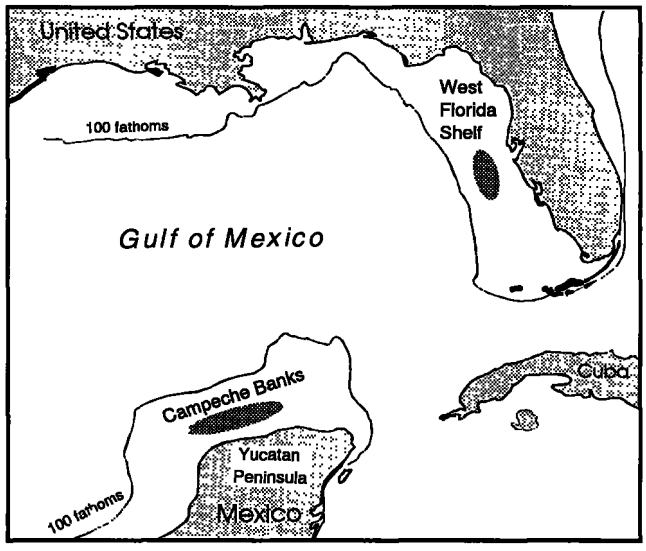

Figure 1
Sampling localities of red grouper (*Epinephelus morio*).

zyme analysis package; McElroy et al., 1992) computer software packages. A minimum-length parsimony network of mtDNA haplotypes was constructed by connecting haplotypes in increments of (inferred) single site gains and losses.

Results

Digestion patterns of the ten restriction enzymes revealed 16 mtDNA haplotypes among all red grouper assayed to date (exclusive of the five individuals from the Dry Tortugas sampled by Richardson and Gold [1993, Table 1]). Of the 16 mtDNA haplotypes, one (haplotype 1) accounted for 77% of all individuals sampled. Four haplotypes were present in both geographic regions. The remaining 12 haplotypes were unique to a geographic region (Table 1). Percentage nucleotide sequence divergence between individual haplotypes ranged from 0.09 to 0.59 (mean ±SE = 0.27 ±0.01).

MtDNA nucleon diversity among individuals from the Campeche Banks was 0.365. This value is lower than that found among individuals from the west coast of Florida (0.457). Percentage intrapopulational nucleotide sequence diversity among individuals from the Campeche Banks was 0.042 ± 0.001 (mean ± SE), as compared with 0.078 ± 0.003 among individuals from the west coast of Florida. Nucleon and intrapopulational nucleotide sequence diversities among all red grouper assayed to date are 0.389 and 0.059 ± 0.001, respectively. Values obtained are based on the 28 restriction enzymes surveyed by Richardson and Gold (1993) and on the assumption that the restriction enzymes previously found to be monomorphic among red grouper from the west coast of Florida are monomorphic among red grouper from Mexico as well.

Results of tests for homogeneity of mtDNA haplotype frequencies between the two localities were nonsignificant (G=20.02, P≈0.21 and χ^2=14.71, P=0.55). The parsimony network (Fig. 2) included a single "assumed" haplotype (i.e. one not detected in the survey). All the haplotypes in the network, including the "assumed" haplotype, could be derived from adjacent haplotypes by one or two restriction site changes. The most common haplotype (haplotype 1) was considered to be central, and nine of the remaining 14 haplotypes were derived from the central haplotype by a single restriction site change. Haplotypes 5 and 8 are most divergent and are separated from the common haplotype by 4 restriction site changes. Except for haplotypes 5 and 8, and 6 and 7 (all from Campeche Banks, Mexico) which are grouped by a single restriction site change, no geographic partitioning was evident.

Table 1
Distribution of 16 mitochondrial DNA composite genotypes (haplotypes).

Haplotype number	Composite MtDNA genotype[1]	Locality	
		Campeche Banks	West Florida Shelf
1	AAAAAAAAAA	43	34
2	AAABAAAAAA	1	1
3	AAAAAAAAAB	3	2
4	AAAABAAAAA	1	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
13	AAAACAAAAA	2	—
14	ACAAAAAAAA	2	—
15	AAAAAAADAB	1	—
16	AAAAAAABAA	1	—

[1] Letters (from left to right) are digestion patterns for *Apa*I, *Kpn*I, *Nco*I, *Nde*I, *Nhe*I, *Nsi*I, *Pvu*II, *Ssp*I, *Xba*I, and *Xmn*I. Restriction fragment sizes may be found in Richardson and Gold (1993). Fragment sizes for three restriction fragment patterns not previously identified are as follows: (in base pairs) *Kpn*I(C): 16800; *Nhe*I(C): 6800, 3200, 2950, 2450, 1300, 50; and *Ssp*I(D): 6000, 5400, 2900, 1700, 800.

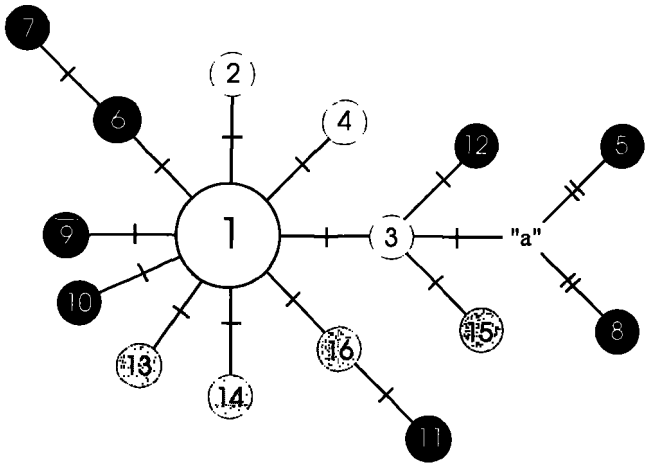

Figure 2

Minimum-length parsimony network of red grouper mitochondrial DNA haplotypes. Numbers refer to mtDNA haplotypes listed in Table 1. Hatch marks represent the number of restriction site changes among individual haplotypes. The haplotype designated by "a" refers to a haplotype assumed to exist, but not detected in the survey. Shaded and solid circles refer to haplotypes unique to a locality (West Florida Shelf and Campeche Banks respectively). Open circles are haplotypes found in both localities.

Discussion

Homogeneity in mtDNA haplotype frequency and absence of phylogeograhic structure among haplotypes are consistent with the hypothesis that red grouper from the west Florida shelf and the Campeche Banks represent a single unit stock. There are caveats to this hypothesis. First, genetic homogeneity does not unequivocally establish occurrence of a unit stock, in part because proof of a null hypothesis is impossible. Genetic homogeneity in this case is simply consistent with the hypothesis that samples are drawn from a single population with the same parametric haplotype frequencies. In addition, small amounts of gene flow are sufficient to homogenize populations genetically (Allendorf and Phelps, 1981), even though geographic samples may be discontinuous demographically. Another caveat is that observed homogeneity may reflect historical rather than current events. Present-day populations could be isolated spatially but have had enough contact in the recent past such that haplotype frequencies are overshadowed by historical gene flow. Examination of a more rapidly evolving nuclear marker (e.g. microsatellite loci) may provide data that suggest such a scenario.

Within-population mtDNA diversity among red grouper from the Campeche Banks was lower than that reported previously for red grouper from the west Florida shelf, and overall, red grouper have among the lowest levels of mtDNA diversity reported for marine fish species (Table 2). Levels of intrapopulational mtDNA diversity are thought to reflect evolutionary-effective population sizes of females (Avise et al., 1988), although there is some evidence (Gold et al., 1994) that intrapopulational mtDNA diversities may also reflect contemporary (female) population sizes as well. The latter is of interest given that some of the species with low intrapopulational mtDNA diversities (e.g. weakfish, orange roughy) have experienced significant reductions in population sizes over the past several years (Graves et al., 1992b; Smolenski et al., 1993). The mtDNA diversity observed in red grouper may thus indicate that red grouper warrant immediate attention in terms of management regulation. Alternatively, red grouper and black sea basses, the species with the lowest reported mtDNA diversities (Table 2), are protogynous hermaphrodites (Manooch, 1988), and it is possible that this mode of reproduction may affect estimates of mtDNA diversity. Estimates for black sea bass (Bowen and Avise, 1990), however, may be somewhat compromised by the low sample sizes, given that a significant proportion of the sampling variance for estimates of nucleotide diversity stems from population sampling (Lynch and Crease, 1990). Further study of mtDNA diversity in other hermaphroditic fishes and in sea bass is clearly warranted.

Present-day gene flow

The observed genetic homogeneity in *E. morio* from west Florida and Mexico was surprising because, a priori, we expected gene flow between the two areas to be minimal and the two populations to be divergent in mtDNA haplotypes. This expectation was based on available information about the life history of red grouper and on reported discontinuity in red grouper distribution. Observations from divers and aquaria personnel have shown that juvenile red grouper are fairly sedentary, preferring to hide in crevices or shells of shallow nearshore habitat (Moe, 1969). Adult red grouper also are important members of the benthic community, frequently occupying crevices, ledges, and caverns formed by rugged limestone reefs. There is, however, evidence that red grouper do migrate at least to some extent, and tagging data suggest "developmental" migration from shallow coastal waters to depths greater than 36 m at approximately 5 years of age (Moe, 1966, 1967;

Table 2

Comparison of estimates of intrapopulational mtDNA diversity in various species of marine fishes.

Species	Number of individuals surveyed	Number of mtDNA haplotypes	Nucleotide sequence diversity (%)
Bluefish[1]	372	40	1.23
Atlantic herring[2]	69	26	1.09
Gulf Menhaden[3]	16	16	0.99
Red drum[4]	693	99	0.88
Spanish sardine[5]	73	24	0.52
Red snapper[4]	421	68	0.50
Black drum[4]	300	37	0.48
Greater amberjack[6]	59	23	0.34
Spotted seatrout[7]	384	73	0.31
Orange roughy[8]	244	22	0.13
Weakfish[9]	370	11	0.13
Red grouper	105[10]	16	0.05[11]
Atlantic black sea bass[2]	19	3	0.03
Gulf black sea bass[2]	9	2	0.03

[1]Graves et al., 1992a.
[2]Kornfield and Bogdanowicz, 1987.
[3]Bowen and Avise, 1990.
[4]Gold et al., 1994.
[5]Tringali and Wilson, 1993.
[6]Richardson and Gold, 1993.
[7]Gold, J. R. 1995. Dep. of Wildlife and Fisheries Sciences, Texas A&M Univ., College Station, TX 77843-2258. Unpubl. data.
[8]Smolenski et al., 1993.
[9]Graves et al., 1992b.
[10]Number of individuals includes 5 additional specimens from the Dry Tortugas surveyed by Richardson and Gold (1993).
[11]Value obtained by using 28 restriction enzymes surveyed in Richardson and Gold (1993). Value obtained from the ten polymorphic restriction enzymes surveyed here is 0.15.

Beaumariage[1]). Presumably, this migration corresponds to the onset of sexual maturity. Other tagging data (Moe, 1966) suggest that adult red grouper may move as much as 18 to 50 miles over a period of time from several months to a year. Finally, on the basis of data from other species of *Epinephelus* (Mito et al., 1967), the pelagic larval stage in *E. morio* is presumed to last 30–40 days, during which larvae are dispersed by ocean currents along a great portion of the Florida shelf (Moe, 1969). However, despite the evidence suggesting that individual red grouper could move considerable distances, consistent patterns of migration in red grouper are not reported, and it is generally presumed that adult red grouper do not undergo large-scale movements offshore.

Gene flow between the west Florida shelf and the Campeche Banks via migration of adults would have to occur either 1) along the north-central and western Gulf or 2) across the Florida Straights. Rivas (1970) noted circumstantial evidence suggesting that there may be seasonal migration of red grouper between the northern and southern Gulf, most probably via a western route. Red grouper, however, are rarely taken in the Gulf west of the Mobile Basin (Springer and Bullis, 1956), and virtually no landings of red grouper occur along most of the Texas coast (Osburn[2]; Campbell[3]). The apparent paucity of red grouper in the northwestern Gulf may reflect either the absence of suitable habitat along the Texas–Louisiana shelf or be a result of some other extrinsic barrier (McEachran[4]). These observations suggest that movement of adult red grouper through the western Gulf is unlikely, if it occurs at all. With respect to movement across the Florida Straights, Rivas (1970) considered it unlikely that red grouper, a bottom dwelling fish, would cross great depths. The Florida Straights are characterized by 100 to 2,000 fathom depths that separate the Campeche Banks from the west Florida shelf (Rezak et al., 1985). This range also suggests limited movement, if any, of red grouper from west Florida to the Campeche Banks.

Alternatively, present-day gene flow among red grouper could occur through dispersal of larvae by ocean currents. Shulman and Bermingham (1995) recently examined variation in mtDNA data among eight species of reef-associated fishes and searched for correlations between gene flow and egg type (pelagic and nonpelagic) and length of planktonic (usually larval) life, two life history traits which could potentialy affect dispersal capability. Although surface currents that might explain observed genetic homogeneity in five of the species were identified, neither egg type nor length of larval stage appeared to be an adequate predictor of geographic structure in reef associated fishes (Shulman and Bermingham, 1995). Therefore, even though red grouper may have

[1] Beaumariage, D. S. 1969. Returns from the 1965 Schlitz tagging program including a cumulative analysis of previous results. Fla. Dept. Nat. Resources, Mar. Res. Lab., Tech. Ser. No. 59:1–38. Div. of Mar. Resources, Dep. of Environmental Protection, Florida Mar. Res. Inst., 100 Eighth Ave. SE, St. Petersburg, FL 33701.

[2] Osburn, H. R. 1988. Trends in finfish landings by sport-boat fishermen in Texas marine waters, May 1974–May 1987. Texas Parks Wildl. Dep., Manag. Data Ser., no. 150, Austin, TX. Fisheries and Wildlife Div., Coastal Fisheries Branch, Texas Parks and Wildlife Dep., 4200 Smith School Road, Austin, TX 78744.

[3] Campbell, R. P. 1993. Trends in Texas commercial fishery landings, 1972–1992. Texas Parks Wildl. Dep., Manag. Data Ser., no. 106, Austin, TX. Fisheries and Wildlife Div., Coastal Fisheries Branch, Texas Parks and Wildlife Dep., 4200 Smith School Road, Austin, TX 78744.

[4] McEachran, J. D. 1995. Dep. of Wildlife and Fisheries Sciences, Texas A&M Univ., College Station, TX 77843-2258. Personal commun.

a lengthy pelagic larval stage, there is no reason to assume a priori that gene flow occurs via dispersal of red grouper larvae. Nonetheless, it cannot be ruled out as a contributing factor.

Historical bottleneck

In a general sense, genetic homogeneity and absence of phylogenetic structure are compatible with limited gene flow under models where isolated populations (or subpopulations) have recently diverged from a panmictic population that possessed low levels of genetic variation and where each subpopulation has a small effective population size. An example of isolated subpopulations that are genetically homogeneous and also genetically depauperate are African cheetahs, where subspecies in east and south Africa are essentially monomorphic for the same alleles at numerous genetic loci (O'Brien et al., 1987). To account for both genetic homogeneity between, and low genetic variability within, subpopulations, O'Brien et al. (1987) hypothesized the past occurrence of at least two genetic bottlenecks. Their hypothesis was based on the premise that genetic homogeneity of isolated subpopulations was consistent with a historical event; whereas low genetic variability in extant populations was consistent with a more recent event.

Red grouper fit the cheetah model in that the subpopulations surveyed are genetically homogeneous and each possesses limited genetic variation. We suggest the possibility that red grouper from west Florida and Mexico are isolated genetically, but that recurring genetic bottlenecks continue to generate high frequencies of the most common genotype. In addition, we suggest that red grouper from these two regions were not isolated historically and that the historical population underwent a severe bottleneck that reduced much of the extant genetic variation. These suggestions account for the observed genetic data and how isolated populations can be genetically homogeneous. A historical bottleneck could have occurred during late Pleistocene times when environmental fluctuations impacted the biota of the region (Rezak et al., 1985; Graham and Mead, 1987). Our suggestions could be tested, in part, by asking whether rare haplotypes found in both locals are identical by descent (i.e. independently derived). In red grouper, two of the three haplotypes shared between west Florida and Campeche Banks are the result of a site loss from the common haplotype and could be the result of a nucleotide substitution at any one of six nucleotide positions. Examination of a more rapidly evolving nuclear marker in individuals from each locality that share these rare haplotypes would address this issue.

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