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RESTRICTION-ENZYME-SITE MAPS OF MITOCHONDRIAL DNA FROM RED SNAPPER (*LUTJANUS CAMPECHANUS*) AND KING MACKEREL (*SCOMBEROMORUS CAVALLA*).—The red snapper (*Lutjanus campechanus*) and king mackerel (*Scomberomorus cavalla*) are two species of considerable economic importance in the Gulf of Mexico (Gulf). Both have experienced population declines over the last decade (GMFMC, 1989; MSAP, 1994), and fisheries for both species are currently regulated to allow stocks to recover. We are using restriction-enzyme-site polymorphism of mitochondrial (mt)DNA to study the spatial and temporal distribution of genetic variability in both species. The purpose of the studies is to determine whether genetic subdivision in either species occurs in the northern Gulf and/or along the Atlantic coast of the southeastern United States (Atlantic). The utility of restriction-site analysis of mtDNA to assess subdivision or stock structure within species has been reviewed extensively (Avise, 1987; Ovenden, 1990; Avise et al., 1987). Part of our work has been published (Camper et al., 1993), and part is in progress.

Because restriction sites cannot always be ascertained solely from restriction fragment patterns, it is often necessary to map restriction sites in order to confirm homology of individual sites. This is of importance when comparisons of mtDNA variation are made among studies from different laboratories. In addition, identification of specific mtDNA restriction sites is useful in cloning and analysis of specific regions of the mtDNA molecule (e.g., the D-loop or control region). In this note we present detailed restriction-enzyme-site maps of mtDNA from red snapper and king mackerel. The maps should prove useful to future genetic studies of both species.

Materials and Methods.—Red snapper were obtained from eight localities across the northern Gulf, from near Port Isabel, Texas to near Panama City, Florida and from near Merida, Mexico. King mackerel were obtained from seven localities across the northern Gulf (from near Port Aransas, Texas to the Florida Keys), from two localities in Mexican waters (near Merida and off the Yucatan peninsula), and from four localities along the southeastern Atlantic coast (from near Daytona Beach, Florida to near Morehead City, North Carolina). Specimens were obtained by angling and from recreational fishermen. Heart and muscle tissues were stored in liquid nitrogen. Type II restriction endonucleases were used in 40-µl reactions to digest 1.0–1.5 µg of genomic DNA following manufacturers specifications. Restriction enzymes used for red snapper included: *Apa* I, *Bcl* I, *Bgl* II, *Dra* I, *Hin*dIII, *Hpa* I, *Nco* I, *Nde* I, *Nhe* I, *Mlu* I, *Pst* I, *Pvu* II, *Sac* I, *Sca* I, *Sma* I, *Stu* I, and *Xba* I. Restriction enzymes used for king mackerel included: *Alu*44I, *Apa* I, *Bcl* I, *Dra* I, *Eco*RI, *Eco*RV, *Hpa* I, *Kpn* I, *Nco* I, *Pvu* II, *Sac* II, *Sph* I, *Ssp* I, *Stu* I, and *Xba* I. Procedures for genomic DNA isolation, agarose electrophoresis, Southern blotting to nylon membranes, hybridization to ^{32}P labeled mtDNA probes, and autoradiography followed Gold and Richardson (1991). Fragment sizes were estimated by fitting migration distances to a least-squares regression line of lambda DNA-*Hin*dIII fragment migration distances. Entire red snapper and king mackerel mtDNA molecules cloned into bacteriophage lambda were used as probes.

Restriction enzyme sites in the mtDNA of both species were mapped using single and double digestions following Brown and Vinograd (1974), and by using polymerase chain reaction (PCR) amplification of known mtDNA genes, as described in Schmidt and Gold (1992). Sequences of primers used may be obtained upon request from the last author. PCR amplifications followed methods outlined in Kocher et al. (1989), using primers designed by T. Schmidt. PCR-amplified products were used in single digestions to localize restriction sites, and were purified and used to probe genomic blots in order to orient individual restriction sites to the mtDNA gene map. For red snapper, sites for the restriction enzymes *Bgl* II, *Nde* I, *Mlu* I, and *Pst* I were mapped and used as reference points to orient other sites. For king mackerel, restriction sites for the enzymes *Sac* II and *Hpa* I were used as references in site orientation. Two *Sac* II sites (one each in the 12S and 16S rRNA genes) and one *Hpa* I site (in an asparagine tRNA gene) are generally conserved among vertebrates (Carr et al., 1987).

Results and discussion.—Totals of 707 red snapper and 678 king mackerel have been surveyed thus far, with 95 and 93 different restriction sites identified, respectively. In both species,

TABLE 1. Locations (in kb) of restriction-enzyme sites in mitochondrial DNA of red snapper, *Lutjanus campechanus*. Conserved sites are indicated by asterisks (*). Daggers (†) identify sites that are not screened routinely.

Restriction enzyme	Map location	Restriction enzyme	Map location	Restriction enzyme	Map location
Nhe I	0.00*	*Sca* I	6.25*	*Stu* I	11.50
Sca I	0.25	*Apa* I	6.50	*Dra* I	12.00*
Dra I	0.50	*Nhe* I	6.50	*Nhe* I	12.00*
Xba I	0.75*	*Apa* I	7.00	*Bcl* I	12.50
*Hin*dIII	1.00	*Nco* I	7.75*	*Bcl* I	12.75
Nhe I	1.00	*Stu* I	7.75	*Nco* I	12.75
Sma I	1.00*	*Hin*dIII	8.00*	*Xba* I	12.75*
Nhe I	1.25*	*Sma* I	8.00*	*Apa* I	13.00
Stu I	2.50	*Xba* I	8.00	*Dra* I	13.00*
Dra I	2.75*	*Bcl* I	8.25	*Stu* I	13.00
Sac I	3.00*	*Nco* I	8.25	*Apa* I	13.50
Apa I	3.25*	*Sca* I	8.50	*Sca* I	13.50
Nde I	3.25†	*Pvu* II	8.75*	*Nhe* I	13.75
Stu I	3.25	*Sca* I	8.75*	*Bcl* I	14.00
Nhe I	3.50	*Sac* I	9.00	*Dra* I	14.00*
*Hin*dIII	4.00*	*Apa* I	9.25	*Hpa* I	14.00
Sac I	4.00	*Sca* I	9.25*	*Nco* I	14.00
Apa I	4.25*	*Xba* I	9.25	*Xba* I	14.00
Sma I	4.50	*Pvu* II	9.50	*Dra* I	14.50
Stu I	4.50	*Stu* I	9.50*	*Dra* I	14.50
Mlu I	4.75†	*Stu* I	9.50*	*Pst* I	14.50†
Pvu II	4.75	*Bcl* I	9.75	*Sca* I	15.00
Dra I	5.25	*Nco* I	9.75	*Stu* I	15.00
*Hin*dIII	5.25	*Xba* I	9.75	*Dra* I	15.25
Stu I	5.25	*Hpa* I	10.25*	*Stu* I	15.75
Hpa I	5.50	*Nhe* I	10.50*	*Hin*dIII	16.00
Nhe I	5.50	*Sca* I	10.50*	*Nhe* I	16.00
Apa I	5.75	*Dra* I	10.75	*Bgl* II	16.25†
Hpa I	5.75	*Stu* I	10.75*	*Hpa* I	16.25
Nhe I	6.00	*Nhe* I	11.00*	*Bcl* I	16.75
Hpa I	6.25	*Sca* I	11.00*	*Sma* I	16.75*
Sac I	6.25*	*Bcl* I	11.25		

the genome size (±SE) of the mtDNA molecule has been estimated to be 16.80 ± 0.20 kilobase (kb) pairs. Map locations of restriction sites in red snapper and king mackerel mtDNA are given in Tables 1 and 2, respectively. Because standard errors for mtDNA fragment sizes are typically 0.25 kb (Avise et al., 1984), sites that mapped within 0.25 kb of each other were assigned the same location. Orientation of restriction sites in the two species relative to the human mtDNA gene map (Anderson et al., 1981) is shown in Figure 1. Because mtDNA gene order and content appears to be conserved across vertebrates (Moritz et al., 1987; Johansen et al., 1990) we assumed that gene order and content of red snapper and king mackerel were the same as in other vertebrates. This was confirmed for restriction sites in regions amplified by PCR.

Sixty sites were found to be polymorphic in red snapper, while 59 sites were found to be polymorphic in king mackerel. In red snapper, two restriction sites for *Dra* I were found to occur within 0.20 kb of one another and were mapped to position 14.50. The site closer to the conserved *Dra* I site at map position 14.00 was found in only eight individuals, whereas the other site was quite frequent. Two *Stu* I sites, both fixed and approximately 0.20 kb apart, also were found in red snapper mtDNA and were mapped to position 9.50. In king mackerel, restriction sites for three different enzymes were found within 0.25 kb of each other. These included three pairs of *Apa* I restriction sites, one pair of *Eco*RV sites, and one pair of *Ssp* I sites. One pair of *Apa* I sites were estimated to be 0.15 kb apart and were mapped to position 6.25. The site closer to the *Eco*RI site at 8.25 was fixed, while the other was found in only three individuals. The second

TABLE 2. Locations (in kb) of restriction-enzyme sites in mitochondrial DNA of king mackerel, *Scomberomorus cavalla*. Conserved sites are indicated by asterisks (*). Daggers (†) identify sites that are not screened routinely.

Restriction enzyme	Map location	Restriction enzyme	Map location	Restriction enzyme	Map location
*Alw*44I	0.00	*Apa* I	6.25	*Stu* I	12.00*
*Eco*RV	0.25	*Dra* I	6.50	*Kpn* I	12.25
Ssp I	0.25*	*Sph* I	6.50*	*Dra* I	12.50
Apa I	0.50	*Stu* I	6.50*	*Eco*RV	12.50
Sac II	1.00†	*Nco* I	6.75*	*Apa* I	12.75
Stu I	1.00	*Alw*44I	7.25	*Apa* I	12.75
*Alw*44I	1.50*	*Bcl* I	7.25*	*Bcl* I	12.75
*Eco*RV	2.00	*Stu* I	7.50	*Eco*RI	12.75
Sac II	2.25†	*Alw*44I	8.00	*Ssp* I	12.75
*Alw*44I	2.50	*Ssp* I	8.00	*Xba* I	12.75*
Dra I	2.50*	*Eco*RI	8.25*	*Apa* I	13.00
Hpa I	2.50	*Apa* I	8.50	*Apa* I	13.00
Hpa I	3.00*	*Bcl* I	8.75*	*Ssp* I	13.00
Sac II	3.00†	*Pvu* II	9.25	*Stu* I	13.25
Stu I	3.25	*Eco*RV	9.50	*Sph* I	13.50*
Apa I	3.50*	*Nco* I	9.50	*Stu* I	13.50
Bcl I	3.50*	*Stu* I	9.50*	*Eco*RV	13.75*
Ssp I	3.50*	*Nco* I	10.00	*Eco*RV	13.75
Ssp I	3.50	*Pvu* II	10.25*	*Dra* I	14.00
Apa I	4.50*	*Apa* I	10.50	*Bcl* I	14.25
Pvu II	4.50	*Bcl* I	10.50	*Bcl* I	14.50
Sph I	4.50	*Dra* I	10.50	*Hpa* I	14.50
Stu I	4.50*	*Xba* I	10.50*	*Kpn* I	14.50*
Pvu II	4.75	*Stu* I	11.00*	*Xba* I	14.50
Bcl I	5.00	*Bcl* I	11.25	*Ssp* I	15.00*
Sac II	5.00†	*Dra* I	11.25	*Hpa* I	15.50
Ssp I	5.25*	*Kpn* I	11.25	*Ssp* I	16.00
Stu I	5.25*	*Alw*44I	11.50	*Dra* I	16.25
Sph I	5.50*	*Hpa* I	11.50	*Alw*44I	16.50
Hpa I	6.00*	*Apa* I	11.75	*Dra* I	16.50
Apa I	6.25*	*Nco* I	11.75	*Nco* I	16.50

pair of *Apa* I sites were estimated to be 0.10 kb apart and were mapped to position 12.75. The site closer to the *Pvu* II site at position 10.25 was found in only one individual, while the other was variable. The third pair of *Apa* I sites were estimated to be 0.20 kb apart and were mapped to position 13.00. Both sites were polymorphic. Both the pair of *Eco*RV sites and the pair of *Ssp* I sites were found to occur within 0.20 kb of one another, and were mapped to positions 13.75 and 3.50, respectively. For both enzymes, the site closer to the *Eco*RI site at 8.25 occurred in all but one individual, while the other site was fixed.

As noted elsewhere (Avise, 1987; Schmidt and Gold, 1992; Avise et al., 1987) the haploidy, maternal inheritance, and rapid evolutionary rate of vertebrate mtDNA make it an ideal genetic tool for studies of genetic stock structure. Studies on stock structure in both red snapper and king mackerel will likely continue in the future, and should be facilitated by the restriction maps presented here. In addition, several laboratories are now working on the culture of red snapper and, given the ease with which mtDNA can be assayed experimentally, mtDNA variants also may prove useful as genetic markers for identifying broodstocks or hatchery-raised individuals.

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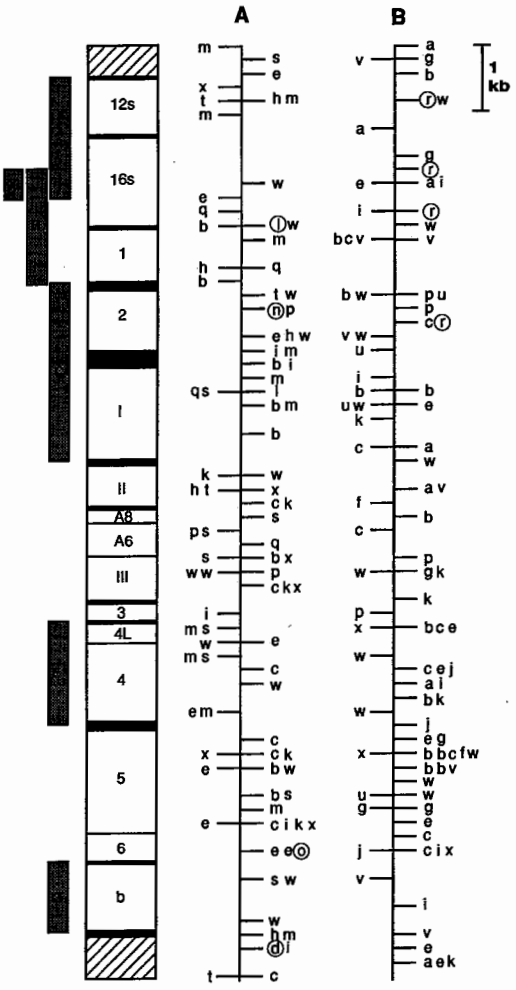

Fig. 1. Restriction-site maps of the mitochondrial DNA of (A) red snapper, *Lutjanus campechanus,* and (B) king mackerel, *Scomberomorus cavalla.* Variable or polymorphic sites are shown to the right of the line, while conserved sites are on the left. Both maps are oriented to the human mtDNA gene map (Anderson et al., 1981): clear boxes refer to the small and large rRNA genes (12S and 16S), NADH dehydrogenase subunits (1–6, 4L), cytochrome oxidase subunits (I–III), ATPase subunits (A6, A8), and cytochrome *b* (b); transfer RNA genes and spacers appear as black areas, and the D-loop or control region is shaded. Size and orientation of regions (boxes A, B, C, E, G, and X below the map) amplified using PCR are also shown. PCR primers were designed and kindly provided by T. R. Schmidt. Enzyme designations are as follows: a, *Alw*44I; b, *Apa* I; c, *Bcl* I; d, *Bgl* II; e, *Dra* I; f, *Eco*RI; g, *Eco*RV; h, *Hind*III; i, *Hpa* I; j, *Kpn* I; k, *Nco* I; l, *Nde* I; m, *Nhe* I; n, *Mlu* I; o, *Pst* I; p, *Pvu* II; q, *Sac* I; r, *Sac* II; s, *Sca* I; t, *Sma* I; u, *Sph* I; v, *Ssp* I; w, *Stu* I; x, *Xba* I. Circles indicate enzymes not routinely screened for polymorphism.

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LONG-TERM MIGRATORY BEHAVIOR OF UNDERSIZED SPINY LOBSTERS *PANULIRUS ARGUS* (LATREILLE) ON THE BERMUDA ISLAND SHELF.—Knowledge of the migratory behavior of an exploited marine species is required for effective management (Harden Jones, 1968; Gregory and Labisky, 1986). Such data facilitates identification of stocks (Cushing, 1968; Gregory and Labisky, 1986). Knowledge of migratory behavior also helps fishermen to increase catch per unit of fishing effort by more effective trap fishing along migration routes (Gregory and Labisky, 1986). Data on population movements therefore not only contribute to the conservation of the stock, but permit more economical use of traps, fuel, and labor.

The movements of a large number of spiny lobsters *Panulirus argus* in South Florida were studied by Gregory and Labisky (1986); the high exploitation rate in Florida resulted in a large proportion of short-term tag recoveries, and consequently the overall pattern of movements described in the paper represented short-term movements taking place during the fishing season (Gregory and Labisky, 1986). Longer-term migrational patterns were not followed.

Short-term migration patterns of *P. argus* lobsters at Bermuda were studied in the 1950s (Sutcliffe, 1952, 1953). The objective of the present study was to determine the longer-term migration patterns of lobsters on the island shelf of Bermuda in order to facilitate the management of the species both there and elsewhere in the Caribbean.

Materials and methods.—The principal tool employed for the trapping and marking work was the traditional 4′ × 4′ × 2′ Bermudan arrowhead trap constructed of 1.5″ hexagonal wire mesh on a spice-wood or rod-iron frame with a single entrance funnel. Undersized animals were marked with numbered Floy spaghetti tags like those used by Davis (1978). The tag was inserted with the injection gun into the abdominal muscle mass (the dorsolateral extensor muscle) just behind the posterior margin of the carapace and slightly to the right of the mid-dorsal line, to miss the gut.

Trapping and tagging of undersized lobsters was carried out at the southwestern and northwestern ends of the island shelf from Aug. 1986 to Sep. 1987. Traps were baited with fish waste and set in "white holes" (sand-floored depressions in the reef) at the northeastern end of the shelf and on the reefs themselves at the southwestern end. Animals were returned to the sea after sexing, measuring, and tagging. Frequently this involved displacing the lobsters between 50 and 200 m as the commercial fishing vessel moved during handling.

Commercial trap fishermen were made aware of the tagging program at a seminar, and cooperated in the return of tags. Information on tag number and date and location of capture was obtained from commercial fishermen during the remainder of the lobster season.

Results.—The records of a tagging study of the movement and dispersal of undersized lobsters (*Panulirus argus*) are shown in Table 1. Females traveled further in 1986–87 (median distance traveled: 12.6 miles) than males (3.5 miles) ($P < 0.05$, Mann-Whitney U-test) and moved faster than males: for females mean ground speed was 0.11 miles/day (standard error, 0.039) and for males mean ground speed was 0.051 miles/day (standard error, 0.018) ($P < 0.10$, t-test). Sample standard deviations were 0.14 and 0.083 miles/day, respectively.

Movements along the depth contour around the atoll system were made in both clockwise and counterclockwise directions around the island shelf. The records of such movements within the field study period (August 1986–September 1987) are separated and detailed in Table 2. The median distances traveled were 13 miles (counterclockwise) and 1.8 miles (clockwise) for six and five animals, respectively. A Mann-Whitney U-test showed that the me-