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SHORT COMMUNICATION

Restriction site heteroplasmy in the mitochondrial DNA of the marine fish *Sciaenops ocellatus* (L.)

J. R. GOLD & L. R. RICHARDSON

Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas, USA

Summary. Restriction site heteroplasmy involving the enzymes *Nco*I and *Xba*I was detected in the mitochondrial DNAs of two individuals of the marine fish *Sciaenops ocellatus*. This represents only the sixth documented example of mitochondrial DNA restriction site heteroplasmy in animals. Two heteroplasmic individuals were found in a survey of nearly 750 individuals, suggesting that in most studies the incidence of mitochondrial DNA site heteroplasmy may be too low to be routinely detected.
Keywords: mitochondrial DNA, restriction site heteroplasmy, marine fish

The mitochondrial genome of animals is typically a compact, circular DNA molecule which is normally assumed to be genetically homogeneous or homoplasmic within single individuals (Brown 1985). Heteroplasmy, the condition where two or more genetically different mitochondrial DNA (mtDNA) molecules coexist within a cell or individual, is rarely found in natural populations. Most documented examples of mtDNA heteroplasmy appear to involve variations in molecule length (reviewed in Nelson 1988). To our knowledge, restriction site heteroplasmy in mtDNA has been documented in only five species: man (Monnat & Loeb 1985), cow (Hauswirth & Laipis 1985), *Drosophila melanogaster* (Hale & Singh 1986), the American shad, *Alosa sapidissima* (Bentzen *et al.* 1988), and the white-footed mouse, *Peromyscus leucopus* (Nelson 1988). The observations in cows and *D. melanogaster* were limited to single maternal lineages, whereas those in *A. sapidissima* and *P. leucopus* involved several individuals from different sample localities (above references). In this note, we report the occurrence of restriction site heteroplasmy in the mtDNAs of two individuals of the marine fish, *Sciaenops ocellatus* (L.) commonly called the red drum.

The two variants were found during a long-term survey of mtDNA restriction fragment length polymorphisms (RFLPs) among red drum sampled from the

Correspondence: J.R. Gold, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843, USA.
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northern Gulf of Mexico and the Atlantic coast of the south-eastern United States. Specific details as to the procedures used in our laboratory are to be published elsewhere, but essentially follow Hillis & Davis (1986) and Maniatis *et al.* (1982). In brief, mtDNA fragment profiles are obtained by digesting purified genomic DNA from each individual with restriction endonucleases. The DNA fragments are separated on 0.8% agarose gels and visualized by autoradiography following Southern transfer to a nylon membrane and hybridization with a ^{32}P-labelled red drum mtDNA probe. A description of the probe may be found in Gold *et al.* (1988). A list of the restriction enzymes employed and the total number of individual mtDNA fragments and fragment patterns resolved to date among nearly 750 red drum surveyed is given in Table 1.

The first putatively heteroplasmic individual was detected among a sample of 23 individuals taken from the Pamlico River near Bath, North Carolina, in the autumn of 1987. The mtDNAs of most of these individuals contained either four (producing the 'B' pattern) or three (producing the 'A' pattern) *Nco*I restriction sites (Fig. 1A, lanes 1 and 4). Comparisons of fragment lengths suggested that *Nco*I-B mtDNA differs from *Nco*I-A mtDNA by the loss of one *Nco*I restriction site. The putatively heteroplasmic individual appeared to contain both *Nco*I-B and *Nco*I-A mtDNAs

Table 1. Restriction endonucleases, total number of fragments, and total number of fragment patterns revealed by each enzyme among approximately 750 red drum *(Sciaenops ocellatus)* examined to date.

	Total number of	
Restriction endonuclease	Fragments	Fragment patterns
*Sca*I	15	11
*Nco*I	14	9
*Bcl*I	13	9
*Spe*I	13	8
*Xba*I	11	7
*Xmn*I	11	7
*Stu*I	10	6
*Pvu*II	9	6
*Eco*RV	9	6
*Eco*RI	6	4
*Pst*I	5†	4
*Hin*dIII	5	3
*Bgl*II	5	3
*Nsi*I	4	3
*Sph*I	2	2
*Bam*HI	1	2*

† Two 16·8-kilobase fragments are not homologous.

* One fragment pattern does not possess a *Bam*HI site.

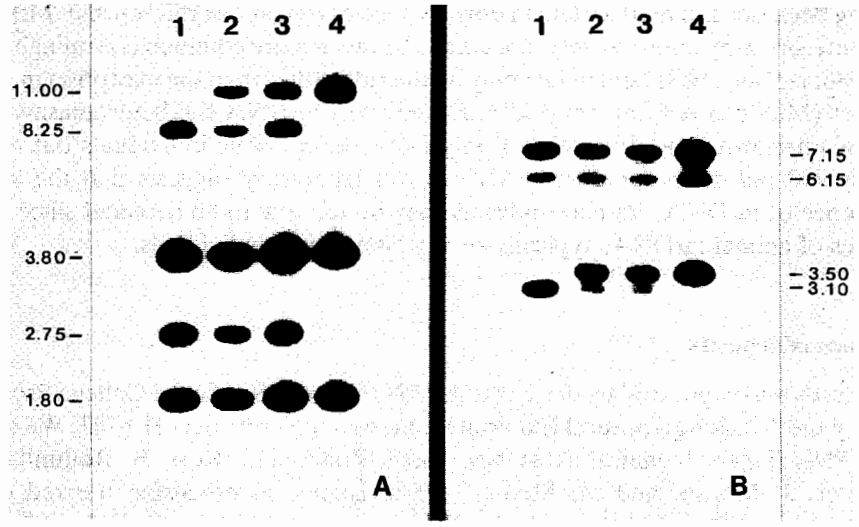

Figure 1. Restriction site heteroplasmy in red drum mtDNA. A: Restriction fragment patterns obtained from *Nco*I digestion. Individuals in lanes 1 and 4 are homoplasmic for *Nco*I-B and *Nco*I-A mtDNAs, respectively. Digestions were carried out using five units of enzyme. Lanes 2 and 3 contain mtDNA from the putatively heteroplasmic individual following digestions with 50 and 25 units of enzyme, respectively. B: Restriction fragment patterns obtained from *Xba*I digestion. Individuals in lanes 1 and 4 are homoplasmic for *Xba*I-D and *Xba*I-A mtDNAs, respectively. Digestions were carried out using five units of enzyme. Lanes 2 and 3 contain mtDNA from the putatively heteroplasmic individual following digestions with 50 and 25 units of enzyme, respectively. A 0·40-kb band generated by the *Xba*I-D genotype is not visible on the autoradiogram. Molecular weights are given in kilobase pairs.

since *Nco*I digests yielded fragments of both types (Fig. 1A). The possibility that the putative heteroplasmy was caused by a length polymorphism was eliminated by the observation that homoplasmic patterns of unit size were encountered with the 15 other restriction enzymes used in our survey. The possibility that the putative heteroplasmy resulted from incomplete digestion was also ruled out since (1) digestions with five and ten times the amount of enzyme normally used still resulted in the heteroplasmic pattern, and (2) the intensity of the 11000-bp band, which is cleaved into smaller bands in *Nco*I-B mtDNA, remained the same following digestions with increased amounts of enzyme (Fig. 1A, lanes 2 and 3).

The second putatively heteroplasmic individual was detected among a sample of 32 individuals taken from Charleston Bay, South Carolina, in the autumn of 1988. The mtDNAs of most of these individuals contained either an *Xba*I-D or an *Xba*I-A pattern (Fig. 1B, lanes 1 and 4). The variant individual appeared to contain both *Xba*I-D and *Xba*I-A mtDNAs since *Xba*I digests yielded fragments of both types (Fig. 1B). The possibility of size heteroplasmy was ruled out in the same way as previously noted, as was the possibility that the putative heteroplasmy stemmed from incomplete digestion (Fig. 1B, lanes 2 and 3).

Our finding of mtDNA restriction site heteroplasmy in the red drum is noteworthy only in that site heteroplasmy appears to be rare in animal mtDNAs,

having been documented to date in only six species (see above references). MtDNA size heteroplasmy, alternatively, appears to be much more common (Bermingham *et al.* 1986; Nelson 1988), and in fact may be the rule rather than the exception in some species (Mulligan & Chapman 1989). Exactly why mtDNA site heteroplasmy is so rare is unknown. The discovery of only two heteroplasmic individuals out of the nearly 750 red drum surveyed to date in our laboratory suggests that the actual incidence of mtDNA site heteroplasmy may be too low to be detected since most studies of animal mtDNAs typically survey 50 or fewer individuals.

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