SHORTER CONTRIBUTIONS: ICHTHYOLOGY

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A TANDEM DUPLICATION IN THE MITOCHONDRIAL DNA OF THE RED SHINER, *CYPRINELLA LUTRENSIS*.—Animal mitochondrial DNA (mtDNA) is relatively conserved with respect to size, gene order, and gene content (Moritz et al., 1987). MtDNA genome sizes range from 15.7 kilobases (kb) in at least two species of sea urchins (Brown, 1981) to greater than 32.1 kb in the deep sea scallop, *Placopecten magellanicus* (Snyder et al., 1987). In most animal species, however, mtDNAs average between 16 and 17.6 kb (Brown, 1985) and contain genes for two ribosomal RNAs, 22 transfer RNAs, and 13 proteins (Moritz et al., 1987).

Length polymorphisms of several hundred to several thousand base pairs have been reported in the mtDNAs of invertebrate and lower vertebrate species. These length polymorphisms are usually characterized as either insertions of relatively short sequences (Monnerot et al., 1984; Harrison et al., 1985; Bermingham et al., 1986), or as tandem repeats of mtDNA sequences (Densmore et al., 1985; Bentzen et al., 1988; Mulligan and Chapman, 1989). Recent studies have shown that the insertions or duplications typically involve one or both ribosomal (rRNA) genes. In addition, the duplicated regions often contain part or all of the control or D-loop region and several protein coding and tRNA genes (Moritz and Brown, 1986, 1987; Wallis, 1987).

In this note, a size variant of approximately 3.6 kb in the mtDNA of the red shiner *Cyprinella lutrensis* (Cyprinidae) is reported. The variant appears to stem from a tandem duplication and to involve the 12S and 16S ribosomal RNA genes as well as the control or D-loop region.

Materials and methods.—The mtDNA size polymorphism was found during a study of geographic variation in mtDNA restriction sites within *C. lutrensis*. The variant molecule was found in one of 12 individuals sampled (by seine) from the Cimarron River in Kingfisher County, Oklahoma.

Specimens of *C. lutrensis* were immediately frozen upon capture in liquid nitrogen and then stored at −80 C. Genomic DNA was isolated from whole fish and digested with various type II restriction endonucleases as per manufacturer's specifications. Restriction fragments were separated in 0.8% agarose gels, transferred to nylon filters (following Southern, 1975), and hybridized to a probe labeled with [^{32}P]dCTP by nick translation (after Rigby et al., 1977). Hybridization conditions, washes, and autoradiography essentially followed Davis (1986). The probe used was the entire mtDNA molecule of *C. lutrensis* cloned in lambda bacteriophage using EMBL arms. Restriction site mapping was carried out by the double digestion method (Sambrook et al., 1989).

Results and discussion.—The variant, representing a 3.6 kb size increase, was detected initially via single restriction enzyme fragment patterns that differed from those normally encountered in *C. lutrensis*. With certain enzymes (e.g., *Bam*HI, *Bcl*I, *Eco*RV, *Hin*dIII, *Nco*I, *Pvu*II, and *Xho*I), a single fragment, usually the largest, was observed that was approximately 3.6 kb larger than "normal" (Fig. 1A). With other enzymes (e.g., *Bgl*II, *Bst*EII, *Eco*RI, *Hpa*I, *Kpn*I, *Nsi*I, *Sca*I, *Sst*I, and *Xba*I), a novel 3.6 kb fragment was observed (Fig. 1B). Finally, with three enzymes (*Apa*I, *Sph*I, and *Sst*II), the smallest fragment usually appeared as a doublet, and a novel fragment was observed that ranged in size from 1.3–2.85 kb (Fig. 1C). These results indicated that the size variation was the result of a tandem duplication in the mtDNA of *C. lutrensis* of approximately 3.6 kb. The first pattern was generated by those enzymes that do not have recognition sites within the duplicated region. The second pattern represents the *prima facie* evidence for the tandem duplication and was generated by those enzymes that have a single recognition site within the duplicated region. The third pattern is explained by the presence of two recognition sites within the duplicated region.

A comparison of the restriction sites inferred to be present within the tandem duplication with our current mtDNA restriction map of *C. lutrensis* (Fig. 2) suggested that the duplication includes the 12S and 16S ribosomal RNA genes. Especially informative was the presence in the duplication of the two *Sst*II sites that are highly conserved in vertebrates (for cyprinids, see Dowling and Brown, 1989) and are known to occur in the 12S and 16S rRNA genes (Carr et al., 1987). To confirm the hypothesis that the duplication includes the rRNA genes, the filters

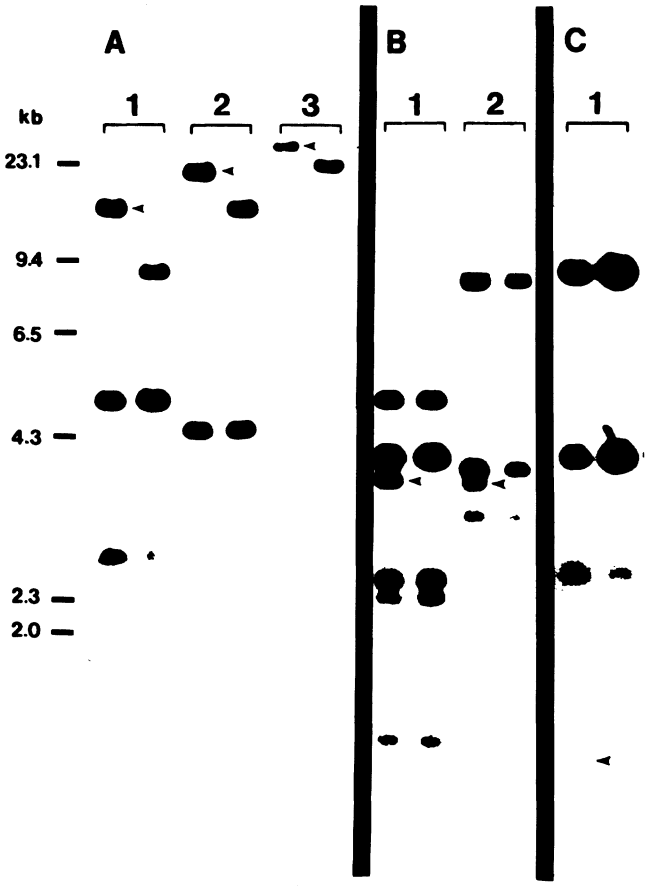

Fig. 1. Evidence for the 3.6 kb tandem duplication in the mtDNA of *C. lutrensis*. (A) Single digestions with *Pst*I (1), *Nco*I (2), and *Bam*HI (3). These enzymes do not have a restriction site within the duplicated region. (B) Single digestions with *Bst*EII (1) and *Hpa*I (2). These enzymes have a single restriction site within the duplicated region. (C) Single digestion with *Apa*I (1). This enzyme is inferred to have two restriction sites in the duplicated region. For each digestion, DNA from the individual with the tandem duplication is on the left; DNA from a "normal" individual is on the right. Arrows indicate novel fragments from the individual with the tandem duplication.

from single digestions with *Bgl*II and *Bst*EII were reprobed separately with two fragments of an rDNA gene clone from the white-tailed deer, *Odeocoileus virginiatus*. One fragment of approximately 850 bp represented part of the 12S rDNA of *O. virginiatus*; whereas the other fragment of about 900 bp represented most of the 16S rDNA gene of *O. virginiatus* (Fig. 2). As shown in Figure 3, the novel 3.6 kb fragment of *C. lutrensis* produced from *Bgl*II and *Bst*EII digestions hybridized to both deer probes. Finally, alignment of the mtDNA gene map of *C. lutrensis* with the map of the closely related species *Cyprinella spiloptera* suggests that the duplication includes both rRNA genes as well as the control or D-loop region, several tRNA genes, and at least part of the cytochrome b and ND1 genes (Fig. 2).

This is the first published report (to our knowledge) of a tandem duplication of this size in a fish. Smaller size variants have been reported in the mtDNAs of the walleye, *Stizostedion vitreum* (Billington and Hebert, 1988); the American shad, *Alosa sapidissima* (Bentzen et al., 1988); the white sturgeon, *Acipenser transmontanus* (Buroker et al., 1990); and the cyprinid *C. spiloptera* (T. E. Dowling, pers. comm.). The occurrence of large size variants in animal mtDNAs, however, has been reported in lizards (*Cnemidophorus*) by Densmore et al. (1985) and

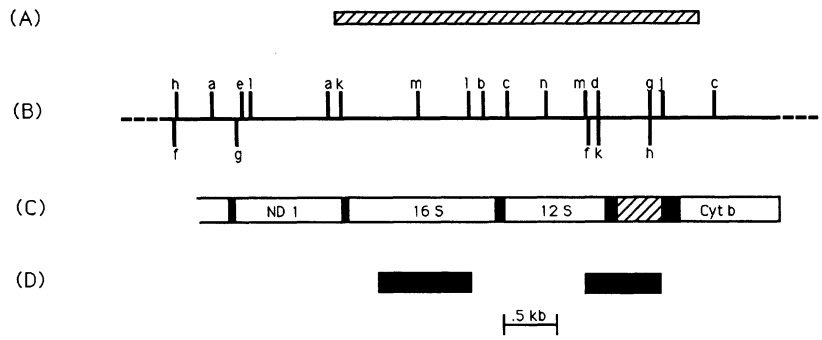

Fig. 2. Restriction site and gene map comparison. (A) Duplicated region inferred from restriction site mapping. (B) Partial restriction site map of the mtDNA of *C. lutrensis*. Enzyme sites are indicated by letters as follows: *Bcl*I, a; *Bgl*II, b; *Bst*EII, c; *Eco*RI, d; *Hin*dIII, e; *Hpa*I, f; *Kpn*I, g; *Nsi*I, h; *Sca*I, j; *Sph*I, k; *Sst*I, l; *Sst*II, m; *Xba*I, n. A complete restriction site map of the mtDNA of *C. lutrensis* may be obtained from the first author. (C) Partial mtDNA gene map from *C. spiloptera*, oriented to the mtDNA restriction site map of *C. lutrensis* using the two *Sst*II sites and the one *Sst*I site that occurs between the two *Sst*II sites in the mtDNA of both species. (D) The approximate sizes and locations of the 16S and 12S rRNA gene probes from the mtDNA of *O. virginiatus*.

may not be entirely unexpected in fishes even though rare (one in >200 individuals surveyed in this study). Our finding that the tandem duplication in the mtDNA of *C. lutrensis* includes the rRNA genes and the control region was not unexpected given the similar findings in other lower vertebrates (Moritz and Brown, 1986, 1987; Wallis, 1987).

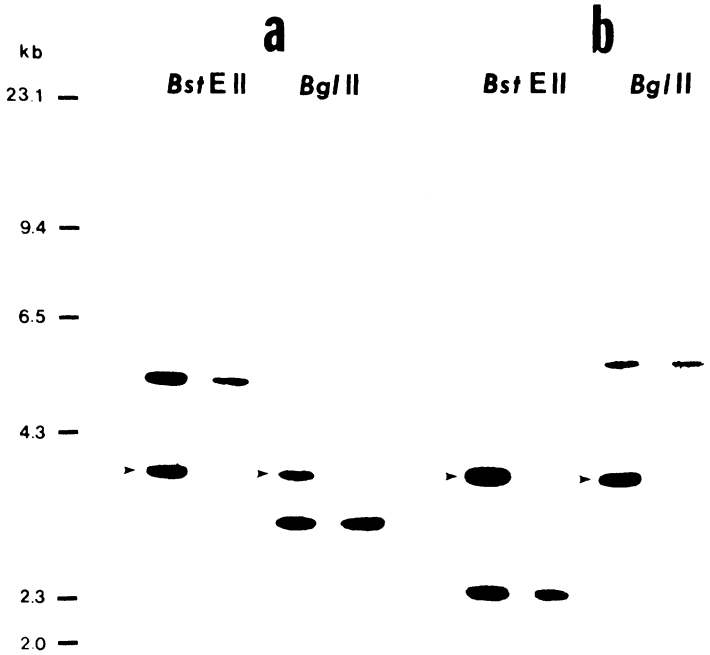

Fig. 3. Evidence that the tandem duplication in *C. lutrensis* includes the rRNA genes. The 3.6 kb novel fragment (arrows) produced by single digestion with *Bst*EII and *Bgl*II is shown to hybridize to the 900 bp fragment of the 16S gene (a) and the 850 bp fragment representing part of the 12S gene (b) from the mtDNA of white-tailed deer (*O. virginiatus*). For each digestion, DNA from the individual with the size variant is on the left; DNA from a "normal" individual is on the right.

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POPULATION STRUCTURE AND DEVELOPMENT OF *LEPIDOGALAXIAS SALAMANDROIDES* (PISCES: SALMONIFORMES) FROM WESTERN AUSTRALIA.—The salamanderfish is a southern hemispheric salmoniform (Williams, 1987). However, it has a very low level of genetic similarity to members of the Galaxiidae and Retropinnidae, sharing only three allelomorphs of 24 loci where comparisons were possible (Berra et al., 1990). It is endemic to ephemeral, acidic waters in southwest