



Mitochondrial DNA Sequence Variation in Domesticated Goldfish, *Carassius auratus*

Richard Beckwitt; Seiji Aoyagi

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tritional source for this particular species of commensal fish. Although the Atlantic bumper apparently does not feed on *Aurelia* medusae, stomach contents of one *H. amblyrhynchus* individual suggest that *Chloroscombrus* may serve as a food source for larger commensal fishes associated with *Aurelia*.

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S. G. TOLLEY, *Department of Marine Science, Uni-*

versity of South Florida, 140 7th Avenue South, St. Petersburg, Florida 33701. Accepted 17 March 1986.

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MITOCHONDRIAL DNA SEQUENCE VARIATION IN DOMESTICATED GOLDFISH, *CARASSIUS AURATUS*.—The mitochondrial DNA (mtDNA) genome of animals has several properties that make it attractive for the study of molecular evolution and systematics. These include: 1) small size—15–19 kilobase pairs (kb); 2) highly conservative gene arrangement; 3) rapid accumulation of point mutations, and to a lesser degree, small additions and deletions; and 4) maternal inheritance (Brown, 1983). The rapid evolution of mtDNA sequences and the maternal pattern of inheritance have proved to be useful in investigating population differentiation and evolution among closely related populations, as well as hybridization between species (Brown and Wright, 1979; Ferris et al., 1983a; Powell, 1983). The literature on mtDNA variation in fishes is limited, but results follow the same patterns observed in studies on other vertebrates (Kessler and Avise, 1985).

In this note, we present preliminary data on mtDNA variation in domesticated goldfish, *Carassius auratus*. The goldfish has a long history of domestication, and most named varieties have been known for at least 500 yr. In addition, wild populations of goldfish still exist in China, and populations of feral goldfish are established in most freshwater drainages in North America, where they hybridize with feral carp, *Cyprinus carpio* (Lin et al., 1969; Taylor and Mahon, 1977; Wang and Li, 1982). The goldfish provides an opportunity to study mtDNA sequence variation after a long period of domestication.

We studied mtDNA sequence variation using restriction enzymes. Restriction enzymes are endonucleases that cut native DNA at specific sequences, usually 4–6 base pairs in length. Thus, the enzyme EcoR I cuts DNA every time the sequence GAATTC appears. Mutations within a restriction enzyme's recognition sequence result in the DNA not being cut at that site. When DNA from two individuals is digested, differences in the numbers and sizes of DNA fragments generated by each restriction

TABLE 1. RESTRICTION ENZYME CLEAVAGE PHENOTYPES FOR *Carassius auratus*. Fragment sizes are in kilobase pairs. Only restriction enzymes that cut the mtDNA more than once are included.

BamH I		Bgl I	EcoR I		Hind III		
A	B	A	A	B	A	B	
11.5	14.5	15.3	7.2	7.2	7.2	6.5	
3.0	0.9	0.7	4.4	4.0	4.8	4.5	
0.9	0.4		2.9	3.3	2.3	2.3	
0.4			1.1	1.1	1.2	1.2	
			0.7	0.7	0.7	1.0	
						0.7	
15.8	15.8	16.0	16.3	16.3	16.2	16.2	(Totals)

enzyme can be related to the number of mutational steps that separate the two sequences.

Methods.—Mitochondrial DNA was purified from ripe ovaries of individual goldfish, using the procedure of Chapman and Powers (unpublished technical report, Maryland Sea Grant Program, UM-SG-TS-85-05). Goldfish, obtained from a commercial distributor in Los Angeles, California, were maintained in the laboratory until killed. Ripe ovaries (0.5–1.0 g per individual) were homogenized on ice in a motor-driven glass and Teflon tissue grinder in 10 ml of TEK buffer (50 mM Tris-HCl, 10 mM EDTA, 1.5% KCl, pH 7.5). The homogenate was layered over 2–3 ml of sucrose-TEK (15% sucrose in TEK), and centrifuged in a swinging bucket rotor at 1000 g for 10 min at 4 C. The supernatant was removed, and recentrifuged as above. This supernatant was then centrifuged at 20,000 g for 30 min at 4 C, the pellet resuspended in 5 ml of TEK and recentrifuged as before. The final mitochondrial pellet was dispersed in 1 ml of 1% sarkosyl in TEK and incubated on ice for 1 h. After centrifugation at 15,000 g for 10 min at 4 C, the supernatant was withdrawn, and the protein removed by extraction with phenol and chloroform-isoamyl alcohol. The remaining aqueous phase was made 0.1 M NaCl, and the nucleic acids collected by precipitation with 2 volumes of cold ethanol. The nucleic acids were pelleted by centrifugation at 15,000 g for 20 min at 4 C, the pellets dried by lyophilization, and the nucleic acids redissolved in 0.1 ml of water. Mitochondrial DNA equivalent to 0.1 g of tissue was digested with five units of restriction enzyme according to the conditions specified by the supplier. Heat-treated RNase A (0.005 mg) was added to each digest to increase the visibility of smaller DNA

fragments. Restriction digests were electrophoresed in 0.7% or 1.0% agarose submarine gels using the TAE pH 8.0 buffer of Maniatis et al. (1982). Gel electrophoresis, staining with ethidium bromide and gel photography were all done using methods from Maniatis et al. (1982). Mitochondrial DNA yields from ripe ovaries were sufficient to observe results from 6–10 digests per individual using ethidium bromide staining. Because yields from other tissues such as liver are many times less, it would be necessary to use radioactive labeling to observe digest patterns. Restriction fragment sizes were estimated using the method of Schaffer and Sederoff (1981). Measurements were made from photographic negatives of gels projected on a large screen to increase accuracy. Restriction fragments of DNA purified from *E. coli* bacteriophage lambda were run on each gel as standards.

Results and discussion.—Mitochondrial DNA samples from 28 specimens were digested with six restriction enzymes: BamH I, Bgl II, EcoR I, Hind III, Sal I, and Xho I (Roberts, 1979). The enzymes Sal I and Xho I each cut the mtDNA only once for each specimen and produced linear fragments of 16.0 kb (SE = 0.2). Two fragments were produced by Bgl II in each specimen; however, there was variation among individuals in the number of fragments produced by three of the other enzymes. Table 1 gives the sizes of the restriction enzyme fragments, including all observed variants. Each individual was scored for its mtDNA phenotype for each restriction enzyme. That is, a given fish may have the "A" phenotype for BamH I, the "B" phenotype for EcoR I, etc. Among the 28 fish examined, three mtDNA phenotypes (combinations of restriction enzyme pheno-

types) were observed. Twenty-three fish had the AAA phenotype (BamH I, EcoR I, Hind III, respectively), three had the ABB phenotype, and two had the BBB phenotype. Although the two BamH I phenotypes can be explained by a single mutational event, at least two mutations are required between the alternate forms of the EcoR I and at least three mutational steps separate the two Hind III phenotypes.

At least three mtDNA clones are present within this sample of goldfish. Nei and Li (1979) introduced the measure nucleotide diversity (π) to quantify the amount of mtDNA sequence variation within a population. In this sample, $\pi = 0.008$. For comparison, in the skipjack tuna *Katsuwonus pelamis* $\pi = 0.002$ (using data for 12 fish from Graves et al., 1984), while in the sunfish *Lepomis cyanellus* $\pi = 0.003$ (using data for 14 fish from Avise and Saunders, 1984). In a sample of 151 *L. macrochirus* including members of two subspecies, only two mtDNA sequences were present, each restricted to one subspecies (Avise et al., 1984). Among mammals, π ranges from 0.002 in humans to 0.01 in *Peromyscus* (Nei and Li, 1979). The amount of mtDNA sequence variation observed in these domesticated goldfish is in contrast to the low levels of variation observed in laboratory strains of the house mouse, where many strains appeared to members of the same clone (Ferris et al., 1983b). This may be explained, in part, by the typical breeding practices of goldfish breeders. Except for fancy breeds raised for show, commercial goldfish are usually the progeny from a large number of matings; as such they may have a widely varied genetic background. This explanation for the observed levels of mtDNA heterogeneity is at variance with the suggestion by Woods and Buth (1984) that high levels of gene silencing observed in nuclear genes of domesticated goldfish, obtained from a similar source, are the result of intense selection or drift associated with a bottleneck during domestication. It remains to be seen whether there is any observable differentiation within or among the morphologically distinct breeds, where selection or population bottlenecks may be presumed to be more intense. These results do suggest that levels of mtDNA heterogeneity in natural populations of the goldfish and related cyprinids will be substantial.

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RICHARD BECKWITT, VANTUNA Research Group, Occidental College, Los Angeles, California 90041 and SEIJI AOYAGI, Biology Department, Occidental College, Los Angeles, California 90041. Present address (RB): Biology Department, Framingham State College, Framingham, Massachusetts 01701. Accepted 19 March 1986.

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A REEVALUATION OF THE KARYOTYPE OF THE ATLANTIC SILVERSIDE, *MENIDIA MENIDIA*.—Proper resolution of fish chromosomes, using modern methods, has become a valuable tool for studies defining genetically isolated populations and for assessing phylogenetic relationships (Rachlin et al., 1978; Turner et al., 1985; Rivlin et al., 1986). Due to the technical limitations of antiquated methods, the older literature on fish karyotypes may be suspect in terms of either the reported diploid complement or chromosome definition by centromeric position. A case in point is the problem with the published chromosomal complement of the Atlantic silverside, *Menidia menidia* (Moenkhaus, 1904). Moenkhaus (1904), examining sections of fertilized eggs of *M. menidia*, determined the diploid chromosome number for this species to be "about 36." In the same study, a chromosome number of 36 is reported for *Fundulus heteroclitus*, a species whose karyotype has been more recently determined to be 48 (Chen and Ruddle, 1970; Fisher and Rachlin, 1972). Therefore, new technological advances in the field of chromosomal analysis and our preliminary findings of a larger diploid

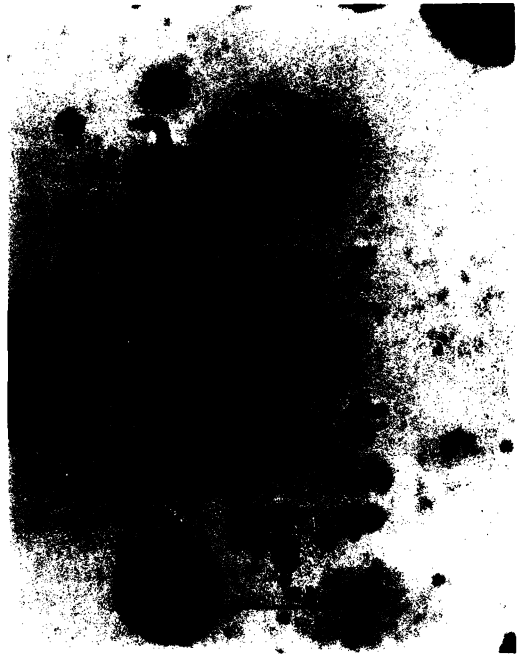


Fig. 1. Metaphase chromosome spread from *Menidia menidia*.

chromosomal number for *M. menidia*, coupled with the fact that Moenkhaus' (1904) methods also underestimated the chromosome number for *F. heteroclitus*, led us to conclude that a re-evaluation of the alpha karyotype of *M. menidia* was appropriate.

In this study adult *M. menidia*, collected from Long Island Sound (40°52'N, 73°47'W), were used for karyotypic analysis. Our karyotypic methods essentially follow those of Rivlin et al. (1985). In order to obtain sufficient metaphase chromosome spreads for analysis, each fish was injected intraperitoneally with 0.1 ml of a freshly prepared 0.1% colchicine solution and maintained in well aerated aquaria for 3 h. The fish were killed by decapitation and the gills removed and placed in a hypotonic solution of 5% KCl for 1.5 h. The gills were then placed in a cold, freshly made solution of 3:1 methanol:glacial acetic acid for 30 min. Three 10 min changes of this solution were made after the initial 30 min treatment. The gills were then held with forceps and dabbed onto clean glass microscope slides and air dried for 24 h. Slides were incubated in 2X SSC (0.3 M sodium chloride and 0.03 M trisodium citrate) at 60 C for 60 min, rinsed in distilled water and stained for 5 min with 4% Giemsa made up in 1/15 M pH

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