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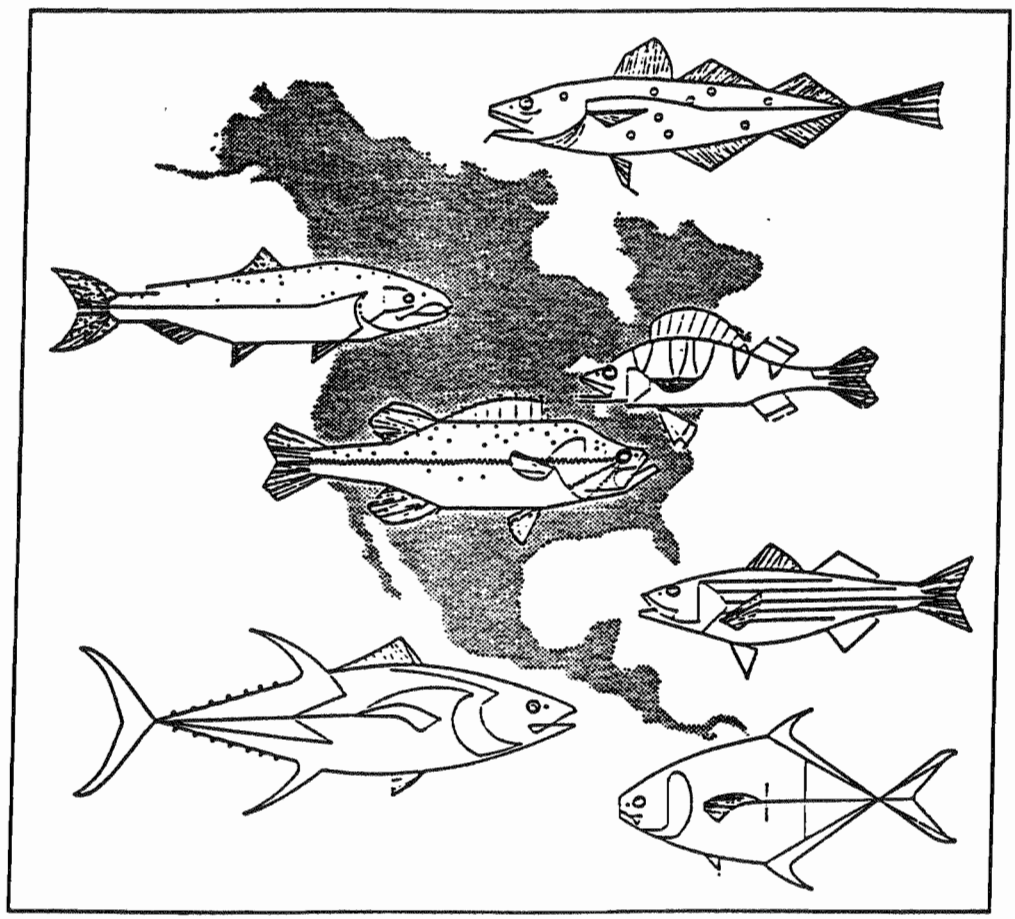

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Natural Occurrence of Triploidy in a Wild Brown Bullhead

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Abstract.—We report the rare, natural occurrence of triploidy in a brown bullhead *Ameiurus* (formerly *Ictalurus*) *nebulosus*. This is to our knowledge the first report of naturally occurring triploidy within the family Ictaluridae. The nuclei of blood cells were studied by flow cytometry, volume measurement, and computer-assisted image analysis. The triploid had a genome size of 3.29 pg DNA/cell compared to 2.23 pg DNA/cell (SD, 0.02 pg) for 28 diploid brown bullheads. Volume measurement and image analysis were able to detect the triploid condition; however, flow cytometry was the most precise technique and had the greatest resolution.

Although the production of triploid fish has become a routine method for creating sterile populations for stocking purposes (e.g., Thorgaard and Allen 1987), the natural occurrence of triploids among vertebrates is rare (Bogart 1980; Schultz 1980). Spontaneously triploid individuals in normally diploid species have been reported among amphibians, reptiles, and birds (e.g., Ohno et al. 1963; Witten 1978; Tiersch and Figiel 1991). Among fish, spontaneous triploidy has been reported in wild and hatchery populations (Cuellar and Uyeno 1972; Gold and Avise 1976; Gold 1986). Dawley et al. (1985) reported triploids resulting from hybridization in natural populations of the sunfish genus *Lepomis*.

Most reports of triploidy are based on karyotypes, but other techniques have been used to identify ploidy. Triploid rainbow trout *Oncorhynchus mykiss* were identified by Thorgaard and Gall (1979) from chromosome counts and nuclear volumes calculated from measurements of Giemsa-stained nuclei. Gold (1986) described a triploid fathead minnow *Pimephales promelas* based on microspectrophotometric analysis of Feulgen-stained nuclei. Coulter counter analysis is used widely in the aquaculture industry to verify ploidy (Wattendorf 1986), and flow cytometry has been used to study the ploidy of fishes (e.g., Thorgaard et al., 1982; Allen 1983) and the genome size of catfish (Tiersch et al. 1990).

This paper documents the occurrence of a triploid brown bullhead *Ameiurus nebulosus* in a natural population. Ploidy was verified by three methods: flow cytometry, Coulter counter, and image analysis.

Methods

Brown bullheads were collected by fyke net from Old Woman Creek, Huron, Ohio. Blood was drawn into vacuum tubes that had been loaded with 0.3 mL of a solution containing sodium citrate (13.2 mg/mL), citric acid (4.8 mg/mL), and dextrose (14.7 mg/mL). Tubes were shipped overnight on ice to Memphis, Tennessee, where samples were stored at 4°C until analysis by flow cytometry and Coulter counter within 3 d of collection.

Nuclear DNA content of blood cells was determined by use of a Coulter Profile flow cytometer (Coulter Electronics, Hialeah, Florida) according to the procedures detailed in Tiersch et al. (1989). Blood cells of hybrid catfish (*Ictalurus punctatus* × *I. furcatus*) were chosen for use as a internal reference in order to avoid overlap of DNA values within a sample. Fresh blood cells from the brown bullheads under study and fresh catfish cells, used as an internal reference, were analyzed as a mixture in 0.5 mL of lysis and staining buffer containing 0.1% sodium citrate, 0.1% Triton X-100,

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TABLE 1.—Cytometric analysis of blood cells of diploid (2C) and triploid (3C) brown bullheads from Old Woman Creek, Ohio. Values are means ± SD; sample sizes (number of fish) are in parentheses. Asterisks indicate that triploid values differ from diploid values at $P < 0.01$** or $P < 0.001$***; other differences are not significant (*t*-tests, $P > 0.05$).

Sample group	Flow cytometry (pg DNA/cell)	Coulter counter			Image analysis		
		Nuclear volume (μm^3)	Cytoplasmic volume (μm^3)	Nuclear: cytoplasmic ratio	Nuclear area (μm^2)	Cytoplasmic area (μm^2)	Nuclear: cytoplasmic ratio
Diploid	2.23 ± 0.02 (28)	13.2 ± 0.9 (5)	104.3 ± 4.5 (5)	0.126 (5)	9.79 ± 1.4 (28)	79.77 ± 8.7 (10)	0.123 (10)
Triploid	3.29*** (1)	18.92** (1)	136.4** (1)	0.139 (1)	16.17*** (1)	86.29 (1)	0.187 (1)
3C:2C ratio	1.48	1.43	1.30	1.10	1.65	1.08	1.52

25 μg RNase (1 mg/mL), and 25 μg of propidium iodide. The DNA content per cell was estimated relative to a value of 7.0 pg per leukocyte nucleus for the human male.

Cell and nuclear volumes for blood cells of the triploid brown bullhead and five diploid brown bullheads chosen at random were measured by use of a Coulter Multisizer equipped with a 70-μm orifice and calibrated with five sizes of latex microspheres ranging from 2.02 to 42.16 μm in diameter. Blood cells were suspended in 10 mL of buffered saline for measurement of cell volume and in saline containing 1 drop of lysing and hemoglobin reagent (Baxter B3157-15) for measurement of nuclear volume. Analysis of at least 20,000 cells or nuclei from each animal was used to generate frequency histograms that supplied mode (peak channel) values corresponding to volume.

Blood smears for image analysis were made in the field with blood collected in EDTA. Slides were air-dried, fixed in 70% methanol for 10 s, and stored until they were stained with Giemsa. Nuclear and cytoplasmic areas for erythrocytes (red blood cells, RBC) were determined by computer-assisted image analysis (Cambridge Quantimet 570). Nuclear and cytoplasmic areas were measured directly on 10 RBC in five fields (50 total) at 400× magnification for 10 randomly chosen diploid brown bullheads and the triploid. Ten nuclear areas per five fields at 400× magnification were also measured for the remaining 18 blood samples.

Results

Of the twenty-nine brown bullheads examined, 28 had a mean nuclear DNA content (±SD) of 2.23 ± 0.02 pg DNA per blood cell. One individual had a value of 3.29 pg DNA/cell, which is 1.48 times greater than the mean value, indicating a triploid (3C) DNA condition (Table 1).

Volumes and measurements of RBC from five diploids and the triploid corroborated the genome size estimates by flow cytometry. Nuclear volume of the triploid was 1.43 times greater than that of the diploids (Table 1). Total cell volume and cytoplasmic compartment volume (total cell volume minus nuclear volume) were each 1.3 times greater for the triploid than for the diploids. The nuclear: cytoplasmic ratio was similar for the triploid and diploids, indicating that the enlarged size of the triploid RBC was due to increased size of both the nucleus and cytoplasm rather than to an increase in a single compartment.

Image analysis of RBC nuclear area distinguished the triploid from the diploids (Table 1). The nuclear: cytoplasmic ratio for the triploid was larger by image than by particle size analysis. If the RBC were flattened with thicknesses of 1.3 μm for diploids and 1.5 μm for the triploid, cellular volumes would equal the values determined by particle size analysis.

Discussion

This is the first report of spontaneous triploidy within the family Ictaluridae. The triploid brown bullhead was the first naturally occurring triploid detected among more than 800 catfish analyzed in the last 4 years by one of us (T.R.T.). In addition, this is one of the few reported cases of triploidy in fish not associated with normally triploid, unisexual populations. It is surprising that naturally occurring polyploidy is not observed more commonly, given the relative ease with which polyploidy can be induced experimentally in catfish and other fishes (e.g., Wolters et al. 1982; Benfey 1989). Image analysis may increase the fre-

quency with which triploidy is detected because sample preparation is simple and the method has no special storage requirements or time limitations.

Flow cytometry directly measures the DNA content of cells, whereas particle size analysis and image analysis are indirect methods of measuring genome size, based on nuclear size. Nuclear size can vary with a number of factors including the age of the cell. Nevertheless, image and particle size analyses appear to provide alternatives to flow cytometry for identifying triploidy. We did not detect mosaicism in the blood sample by any method, but cannot reject the possibility that diploidy or mosaicism existed in other tissues of the triploid fish.

Although our data cannot identify cause, triploidy could have been due to fusion of a haploid ova with either a diploid sperm or two sperm. Polyspermy has been reported in fish (Ginsburg and Stefanov 1990). However, a more likely cause is fusion of a haploid sperm with a diploid ova. Heat, cold, and pressure have all been used experimentally to generate diploid ova via retention of the second polar body. Triploids can be induced routinely in this manner for aquacultural purposes in a number of species (Thorgaard and Allen 1987). It is possible that natural conditions could have produced diploid ova.

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