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Identification of Walleye x Sauger Hybrid by Isozyme Electrophoresis†

J.E. Fulton,‡ J.S. Otis and K.S. Guise

Abstract

Of 125 phenotypic walleye screened by isozyme electrophoresis, one unusual individual was detected and subsequently suspected of being a walleye (*Stizostedion vitreum vitreum*) x sauger (*S. canadense*) hybrid. The isozyme pattern obtained for L-iditol dehydrogenase (IDDH, E.C. 1.1.1.14), phosphoglucomutase (PGM, E.C. 5.4.2.2) and a fast migrating aspartate aminotransferase (AAT, E.C. 2.6.1.1) isozyme showed that this individual had both walleye and sauger isozymes. Isozyme analyses is a useful technique for distinguishing walleye x sauger hybrids from parent species. This is the first report of alleles of the AAT* locus being species specific for sauger and walleye, and the first confirmed report of naturally occuring walleye x sauger hybrids in Minnesota.

Introduction

The occurrence of rare natural interspecific hybrids between walleye (*Stizostedion vitreum vitreum*) and sauger (*S. canadense*) was first reported by Stroud (1948) and later by Clayton et al. (1973) and Nelson and Walburg (1977). Hybrid fish were generally identified because they shared morphological traits with both walleye and sauger. Malate dehydrogenase (MDH) isozyme differences occur between the two species. Sauger are fixed for an extremely fast variant of the MDH-1 isozyme, and lack polymorphism for MDH-3, whereas walleye do not have the fast variant of the MDH-1 isozyme and are polymorphic for MDH-3 (Clayton et al. 1973; Todd, 1990).

Billington et al. (1990) compared isozyme patterns between walleye and sauger and identified four isozymes, L-iditol dehydrogenase (IDDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM) and glutamate pyruvate transaminase (GPT, E.C. 2.6.1.2) as being species diagnostic. In addition, Billington et al. (1988) reported sauger mitochondrial DNA from 2 fish that were indistinguishable from walleye by morphological traits or by isozyme analyses.

The frequency of naturally occurring walleye x sauger hybrids in the waters of southern Manitoba and northwest Ontario has been estimated at 1 % (Clayton et al. 1973). Walleye x sauger hybrids are known to be fertile and have been successfully raised commercially (Nelson et al. 1965, Lynch et al. 1982, Hearn 1986). In a naturally occurring walleye x sauger hybrid, we have confirmed species specificity for *IDDH** alleles. In addition, a new species specific isozyme, a slowly migrating AAT*, is described.

Materials and Methods

Twenty-five walleye were collected from each of 5 locations within Minnesota; Lake of the Woods (Rainy River), Lake Vermilion (Pike River) , Lake Saganaga (Seagull River) , Lake Winnibigoshish and Lake Pepin, either by trap during spawning runs, electroshocking or trawling. All individuals appeared to be walleye by preliminary morphological examinations. Ten sauger were collected from Lake Pepin by trawling. White muscle and liver tissue were removed from freshly killed fish and frozen in dry ice/methanol, shipped on dry ice and subsequently stored at -70 °C. About 0.5 g of each tissue was ground with an equal weight/ volume of 0.1 % solution of 2-mercaptoethanol in H_2O. Samples were spun at 20,000 g for 10 min at 4 °C. Supernatant solutions were removed and stored at -20 °C until used.

Isozymes were separated on cellulose acetate (Helena Laboratories, Beaumont, TX) at 200 V for 25 min. Buffers and stain recipes were modified from Richardson et al. (1986). Nomenclature for enzyme names is as suggested by Shaklee et al. (1989) with the Enzyme Commission (E.C.) numbers (IUBCN, 1984).

IDDH: L-iditol dehydrogenase, E.7. no. 1.1.1.14:

Stain; 1.5 mL 0.1 M Tris(hydroxymethyl) aminomethane (Tris) pH 8.0, 3 mg nicotinamide adenine dinucleotide (NAD), 100 mg sorbitol, 2 mg 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), 0.12 mg phenazine methosulfate (PMS).

Buffer; 0.05 M TM pH 7.8 (0.05M Tris; 0.02 M maleic acid adjusted to pH 7.8 with maleic acid).

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PGM: Phosphoglucomutase, E.C. no 5.4.2.2:

Stain; 1 mL 0.1 M Tris pH 8.0, 3 mg NAD, 0.2 mL 0.5 M $MgCl_2$ 5 mg glucose-1-phosphate grade I, 5 mg glucose-1-phosphate grade VI, 1 U glucose-6-phosphate dehydrogenase, 2 mg MTT, 0.12 mg PMS.

Buffer; 0.01 M PC pH 6.4 (0.01 M Na_2HPO_4, 0.0025 M citric acid, adjusted to pH 6.4 with citric acid).

AAT:Aspartate amino transferase, E.C. no. 2.6.1.1:

Stain; 1.5 mL 0.1 M sodium phosphate buffer, 0.5 mg pyridoxal-5-phosphate, 10 mg L-aspartic acid, 10 mg α-ketoglutaric acid (adjusted to pH 7.4), 0.4 mL fast blue BB salt solution (25 g fast blue BB salt in 15 mL H_2O.

Buffer; 0.02 M phosphate pH 7.0 (0.0116 M Na_2HPO_4, 0.0084 M NaH_2PO_4, adjusted to pH 7.0 with 10 M NaOH).

MDH: Malate dehydrogenase, E.C. no. 1.1.1.37:

Stain; 1 mL 0.1 M Tris pH 8.0, 3 mg NAD, 0.14 M malic acid in 0.1 M Tris pH 8.0, 2 mg MTT, 0.12 mg PMS.

Buffer; 0.015 M TEMB pH 7.8 (0.015M Tris, 0.005M Na_2EDTA, 0.01 M $MgCl_2$, 0.0055 M boric acid). IDDH was obtained from liver tissue and PGM, AAT and MDH were obtained from muscle tissue.

Results

The isozyme analysis of the 5 walleye stocks (124 individuals) screened for 26 isozyme loci has been reported (Fulton et al 1992). One individual was excluded in that analysis because it showed unique isozyme patterns. It was the only individual heterozygous at the *IDDH** locus. It was also the only individual heterozygous at the *AAT** locus and it was one of two heterozygous for the *PGM** locus. Thus, it was the only individual heterozygous for three loci with alleles that were segregating with less than a 1 % frequency in the walleye sample. All 10 sauger were homozygous for the *PGM** and *IDDH** loci. They were also all homozygous for the slowly migrating isozyme of the *AAT** loci and for two *MDH** loci (*MDH-2** and *MDH-3**). All walleye were homozygous for non-sauger allelic forms for *IDDH** and the fast allele of the *AAT** loci. One walleye was heterozygous for *PGM** and the *MDH-3** locus showed segregation of 3 alleles.

Figure 1 shows the electrophoresis patterns and subsequent enzyme specific staining of isozymes obtained from tissues from a representative walleye (lane 1) and sauger (lane 3) and the unusual individual (lane 2; Figs. 1 a, b and c). The anomalous individual has a phenotype consistent with that of a hybrid between walleye and sauger for IDDH (1a), PGM (1b) and AAT (1c) because the samples show isozyme bands from both presumed parental species. Figure 1d shows the isozymes of the *MDH-3** locus. Lane 1 is from a walleye homozygote, lane 2 is from a heterozygous walleye, lane 4 is from a sauger and lane 3 is from the walleye x sauger hybrid. The *MDH-1** locus is species diagnostic for walleye and sauger, with sauger being homozygous for a very fast allele which is not found in walleye (Clayton et al. 1973; Todd, 1990). This *MDH-1** isozyme did not resolve on the cellulose acetate and is not seen in the figure. The *MDH-2** locus is monomorphic while the *MDH-3** locus segregates in the walleye populations tested. All sauger tested were homozygous for the *MDH-3** allele shown in Figure 1d. The MDH isozyme pattern is consistent with the unusual individual being a hybrid.

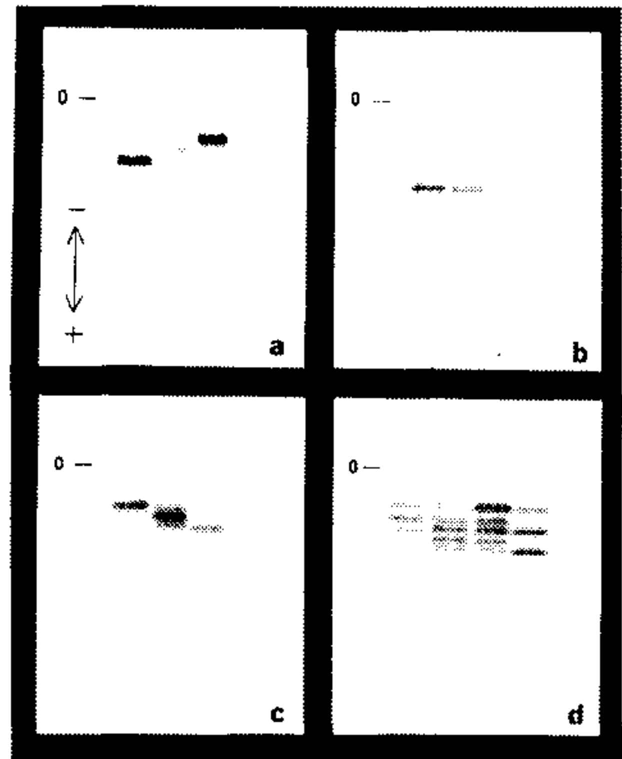

Figure 1. Walleye, walleye x sauger hybrid and sauger isozyme patterns obtained by cellulose acetate separation for IDDH (a), PGM (b), AAT (c) and MDH (d). In a, b, and c, lane 1 is from walleye, lane 2 is from the walleye x sauger hybrid and lane 3 is from sauger. In d, lane 1 is walleye, lane 2 is walleye (MDH-3* hetero-zygote), lane 3 is walleye x sauger and lane 4 is sauger. (O = origin; lane 1 left; lane 4 right)

IDDH is a tetrameric molecule (Richardson et al. 1986), and therefore heterozygotes are expected to have a five-banded pattern consisting of both parental bands and heteromorphic intermediate bands. However, it is often difficult to resolve all five bands because of differing relative intensities of expression. PGM is a monomorphic molecule (Richardson et al. 1986), and therefore both parental forms are found

only in heterozygotes. Rare PGM heterozygotes have been previously detected in walleyes (Todd 1990, Fulton et al. 1992). Because AAT is a dimeric molecule (Richardson et al. 1986), heterozygotes are expected to have a three-banded pattern, consisting of both parental bands and an intermediate heteromorphic band.

All 124 walleye screened were monomorphic at the *IDDH** locus and only one individual was heterozygous at the *PGM** locus. The *AAT** locus was heterozygous in two walleye, however this rare allele in walleye was not the same allele as that found for the sauger as shown by the different isozyme migration patterns. Occurrence of rare heterozygotes is expected, but the probability of several rarely polymorphic loci being heterozygous in one individual is very small.

Since heterozygosity at both the *PGM* and MDH-3** loci has been seen within walleye populations, neither of these two loci can be used to distinguish walleye vs sauger individuals. However, the IDDH* isozyme and the fast migrating AAT* isozyme appear to be species-specific for sauger and, thus, are valuable for species identification.

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