Yellow (*Perca flavescens*) and Eurasian (*P. fluviatilis*) perch distinguished in fried fish samples by DNA analysis

Rex M. Strange

Carol A. Stepien (contact author)

Email address for C. A. Stepien: Carol.Stepien@utoledo.edu

Great Lakes Genetics Laboratory
Lake Erie Center and Department of Environmental Sciences
The University of Toledo
6200 Bayshore Road
Toledo, Ohio 43618

DNA techniques are increasingly used as diagnostic tools in many fields and venues. In particular, a relatively new application is its use as a check for proper advertisement in markets and on restaurant menus. The identification of fish from markets and restaurants is a growing problem because economic practices often render it cost-effective to substitute one species for another. DNA sequences that are diagnostic for many commercially important fishes are now documented on public databases, such as the National Center for Biotechnology Information's (NCBI) GenBank.[1] It is now possible for most genetics laboratories to identify the species from which a tissue sample was taken without sequencing all the possible taxa it might represent.

We were contacted by reporters from a news agency, who were interested in determining whether yellow perch "fish fries" in their local restaurants were, in fact, local yellow perch (*Perca flavescens* Mitchell) taken from the Great Lakes. In recent years it has become economically desirable to substitute Eurasian perch (*P. fluviatilis* Linnaeus) or some other species for yellow perch because of the decline of stocks of yellow perch in the Great Lakes and because of rising prices, both of which raise truth-in-advertising questions. Such cases of substitution usually involve replacement with less expensive fish species, which are difficult to detect in fillets (see Ward, 2000).

We agreed to attempt to identify the fish using DNA techniques because the Great Lakes Genetics Laboratory has several on-going projects involving evolutionary genetics of yellow perch and other percids (e.g., Faber and Stepien, 1997; Ford and Stepien, 2004). However, instead of the fresh frozen fillets that we expected, the reporters sent us breaded and deep fried fish fillets taken from served dinners. We decided to attempt to identify the species of the fish, despite our initial misgivings as to whether useful amounts of DNA could be extracted from deep-fried material. In the present study, we outline a procedure with molecular tools and an analysis that allows the identification of any species for which sequences are documented on GenBank.[1]

Materials and methods

We removed approximately 10 mg of muscle tissue from below the breading of each fried fillet, submerged the tissue in an ethanol (95%) wash, and allowed the wash to sit overnight at room temperature. No other attempt was used to remove any residual cooking oil. Samples were then air-dried before digestion with proteinase K. After complete digestion, DNA was isolated by using standard phenol-chloroform extractions, alcohol precipitation, and two washes with 70% ethanol. From experience, we found that the final pellet of DNA was smaller than the DNA yield we would expect from uncooked material; therefore we suspended the pellet with 30 µL (microliters) of dd (deionized distilled) H_2O rather than the 100 μL we typically use.

The polymerase chain reaction (PCR) was used to amplify a fragment (approximately 400 base pairs) of the mitochondrial cytochrome *b* gene with the following universal primers described by Palumbi (1996): L14724 (5′-GTG ACT TGA AAA ACC ACC GTT G-3′) and Kocher et al. (1989): H15149 (5′-TGC AGC CCC TCA GAA TGA TAT TTG TCC TCA-3′). We chose this particular fragment for two reasons: 1) cytochrome *b* is commonly used in systematic studies of fishes and a wide variety of fish taxa are documented in the GenBank[1] data base, including percids (e.g., Song et al., 1998; Near, 2002; Sloss et al., 2004); and 2) our previous experience has shown that PCR amplification of smaller fragments is often more successful than amplification of larger fragments, especially when dealing with degraded samples such as extracts from deep-fried fillets.

The PCR mixture consisted of a total volume of 50 μL, with concentrations of 1.5 mM (milli Molar) $MgCl_2$, 1.0 μM (micro Molar) of each primer, and 1.0 U (units) of *Taq* polymerase. Amplification parameters consisted of an initial denaturation at 94°C for 2.5 min, followed by 35 cycles of denaturation (94°C, 1 min), primer annealing (52°C, 1 min), and polymerase extension (72°C, 1 min). A final extension at 72°C for 7 min was included to reduce the number of partial strands. Amplification products were then purified by running the entire product

[1] GenBank. 2006. National Center for Biotechnology Information (NCBI), National Institutes of Health, 8600 Rockville Pike, Bethesda, MD 20894. Website: http://www.ncbi.nlm.nih.gov (accessed 14 February 2006).

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on a 2% agarose gel in TAE (Tris-acetate-EDTA, p.H. 8.0) buffer. The band was made visible with ethidium bromide staining and was then excised and purified in a spin column. The resulting gel-purified PCR product was then used as a template for another round of PCR with identical parameters. Samples consisting of 250 ng (nanograms) of purified PCR product and 16 pmol (pico moles) of primer (L14724) were sequenced on an automated ABI 3700 sequencer (Applied Biosystems Inc., Fullerton, CA).

Because we did not know the identity of the species in the fish fillets, each sequence was submitted to a BLAST (basic local alignment search tool) search on the NCBI GenBank[1] database, which provides the identification of species by sequences. After we were confident that the identities of the species in the fillets were limited to the genus *Perca*, the sequences were aligned sequentially with cytochrome *b* sequence data from all three species of *Perca* (e.g., *P. flavescens*, *P. fluviatilis*, and *P. schrenkii*), and two outgroup percid taxa (walleye [*Sander vitreus*] and ruffe [*Gymnocephalus cernuus*]) by using the computer program CLUSTALX2[2] (Thompson et al., 1997). A tree of the relationships among the sequences was constructed by using maximum likelihood estimates of sequence divergences with a neighbor-joining network (Saitou and Nei, 1987) as implemented in the computer program PHYLIP (PHYLogeny Inference Package; Felsenstein[3]).

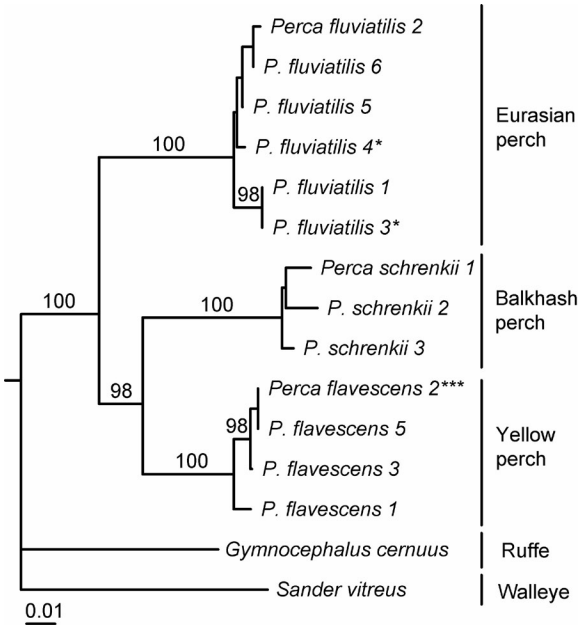

Figure 1

Phylogenetic relationships among *Perca* and outgroup mtDNA haplotypes as inferred from cytochrome *b* sequence divergences. Taxon labels are consistent with those in Table 1. * = sequence match to an individual fried fish fillet sample sequenced in this study. Numbers at nodes indicate percent support for the relationships from 1000 bootstrap pseudoreplications.

Results and discussion

Our extraction procedure yielded DNA usable for PCR and subsequent sequencing, although the extractions were degraded and of relatively low molecular weight. Thirteen complete cytochrome *b* sequences representing all three species of *Perca* were found documented on GenBank[1] (Table 1). Yellow perch is one of three described species of this genus, for which a substantial fishery exists in the Great Lakes, although fish stocks have declined at some localities.[4] The natural distribution of yellow perch extends from Nova Scotia south along the Atlantic coast of North America to South Carolina, and west to Montana (Scott and Crossman, 1973; Craig, 2000). The Eurasian perch is very similar morphologically to *P. flavescens*, is found throughout most of northern Europe and Asia (Craig, 2000; Maitland, 2000), and also represents a commercially important species. A third species, *P. schrenkii* Kessler, is restricted to the eastern portion of Kazakhistan and does not contribute to the world market.

Each species of *Perca* is represented by unique nucleotide cytochrome *b* sequences and has marked divergences (Fig. 1). Three of our five samples matched one of the cytochrome *b* sequences of the yellow perch *P. flavescens* (designated as *flavescens* 2), and two matched sequences of the Eurasian perch *P. fluviatilis* (denoted as *fluviatilis* 3 and *fluviatilis* 4). It is noteworthy that large genetic divergences separate the mtDNA cytochrome *b* sequences of *P. flavescens* and *P. fluviatili*s. These two species differ at 130 sites (11.4%; Song et al., 1998; Sloss et al., 2004), and intraspecific variation for each is an order of magnitude less (Fig. 1; also see Billington, 1993). Thus, it is unlikely that our identifications were in error.

Although one would expect that most of the perch fillets at local markets are caught locally, the supply of and demand for North American and Eurasian perch determines which species is the most economical to serve. Importers and exporters trade fish from both sides of the ocean and the price fluctuates seasonally for both species. Winter is the low supply period for both,

[2] Gibson, T., D. Higgins, J. Thompson, and F. Jeanmougin. 2006. ClustalX. Plate-forme de bio-informatique (Bioinformatic platform), I.G.B.M.C., 1 rue Laurent Fries, 67404 Illkirch, Cedex, France. Website: http://bips.u-strasbg.fr/fr/Documentation/ClustalX (accessed 14 February 2006).

[3] Felsenstein, J. 1995. Department of Genome Sciences, University of Washington, Box 357730, Seattle, Washington, USA 98195-7730. Website: http://evolution.genetics.washington.edu/phylip/general.html (accessed 20 February 2006).

[4] Johnson, T. B. 2006. Personal commun. Ontario Ministry of Natural Resources, Glenora Fisheries Station, R.R. #4, 21 Hatchery Lane, Picton, Ontario, Canada K0K 2T0.

Table 1

Taxonomic assignment and GenBank (Footnote 1 in the main text) accession numbers for each of the diagnostic mitochondrial cytochrome *b* sequences used in our comparisons with unknown fried fish fillets. Sequences from ruffe (*Gymnocephalus cernuus*) and walleye (*Sander vitreus*) were used for outgroup comparison. Taxonomic labels are consistent with the labeling on the tree in Figure 1. Each asterisk denotes that a single fried fish fillet sample was matched to this known sequence. * = one sample matched; *** = three samples matched.

Taxon	Haplotype designation	GenBank accession number
Yellow perch	*Perca flavescens* 1	AY374280
	P. flavescens 2***	AF546115
	P. flavescens 3	AF386600
	P. flavescens 4	AF045357
Eurasian perch	*Perca fluviatilis* 1	AY374281
	P. fluviatilis 2	AF546117
	P. fluviatilis 3*	AF546116
	P. fluviatilis 4*	AY929376
	P. fluviatilis 5	AF386599
	P. fluviatilis 6	AF045358
Balkash perch	*Perca schrenkii* 1	AF546120
	P. schrenkii 2	AF546119
	P. schrenkii 3	AF546118
Ruffe	*Gymnocephalus cernuus*	AF386598
Walleye	*Sander vitreus*	AF386602

but prices quickly fall as the ice clears from the Great Lakes.[4] In North America, the fishery in Lake Michigan (near Milwaukee, Wisconsin) crashed by 2000 and most perch taken in the past from the Great Lakes are presently taken from Lake Erie.[4,5,6]

Lake Erie itself is divided into four quota zones across both U.S. and Canadian waters, of which Zone 2 (comprising the west-central basins) has the largest U.S. allowable quota.[4,5,6] Most of those perch are landed and processed in Wheatley, Ontario, where the largest processing plants are located.[4,6] Current landed prices for North American yellow perch are about Can $2.00/lb, whereas the market price for processed fish runs about Can $8–9/lb.[4] Eurasian perch have been sold in North American markets for over 20 years, and presently are cheaper than North American yellow perch, selling for Can $2–3/lb.[4] Thus, restaurants in the Great Lakes area that advertise yellow perch "fish fries" may be unlikely to offer economical all-you-can-eat dinners and stay in business without imported Eurasian perch. Our results illustrate that use of mtDNA sequencing is an economic and effective method to identify the species in fillets for perch (and other species), even in the case of genetic material that has been deep fried.

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