SHORT COMMUNICATION

GENETIC POLYMORPHISM OF TETRAZOLIUM OXIDASE IN WALLEYE POLLOCK¹⁾

MUNEHICO IWATA

Faculty of Fisheries, Hokkaido University, Hakodate 040

Received February 20, 1973

Intraspecific variants of tetrazolium oxidase (TO) have been demonstrated electrophoretically in man (Brewer 1967) and dogs (Baur and Schorr 1969). Johnson *et al.* (1970) reported interspecies variations of TO in fifteen species of rockfish and suggested a correlation between the time of spawning and tetrazolium oxidase polymorphism. Utter (1971) detected the differences of TO phenotype among rainbow trout and Pacific salmon, and also observed gene frequencies for rainbow trout sampled over a broad geographic range. Recently Numachi (1972) has separated two populations of black rockfish on the basis of differences in TO phenotypes.

This paper describes the electrophoretic phenotypes of the enzyme TO in the walleye pollock (*Theragra chalcogramma*). This research is a part of the investigations seeking methods to recognize different pollock populations.

Three groups of walleye pollock were sampled from the southern coasts in Hokkaido and Pacific coast of Aomori prefecture, including 94 specimens from Kumaishi (in March 1972), 60 specimens from Sahara and 96 specimens from Hachinohe (both in January 1973). Pollock captured by long-line off Kumaishi were rapidly frozen within one hour and stored at -20°C . From Sahara fresh fish were brought to the laboratory within four hours after being captured during commercial fishing operations. Ninety six brains, removed from frozen fish, were transported from Hachinohe to the laboratory, in dry-ice. Tissues examined, were liver, brain, eye, heart muscle, pyloric caecum, kidney, gonad, and skeletal muscle. For comparison with the zymograms of frozen specimen, liver and brain extracts of 16 fresh specimens from Sahara were analysed, immediately. The identification with zymogram from the frozen tissues became gradually difficult after ten days.

A sample tissue was homogenized with distilled water (1:1) and centrifuged for 20 minutes at 20,000 g. The supernatants, absorbed into filter papers (4×6 mm, TOYO No. 2), were analyzed by starch gel electrophoresis using a discontinuous buffer system as described by Ridgway (1970). A constant current of 1.5 mA/cm² was applied for 9 hours throughout the electrophoresis. A sliced gel was steeped in reaction mixtures for visualization. TO activity bands appeared as achromatic regions against non-specific

1) Contribution No. 64 from the Research Institute of North Pacific Fisheries, Faculty of Fisheries, Hokkaido University. Supported in part by a grant in aid for the Japanese Fisheries Agent special science program.

dark blue background after about 2 hours (Fig. 1). The reaction mixture detecting best contrast contained 20 mg nitroblue-tetrazolium and 14 mg phenazine methosulfate per 100 ml distilled water. Preliminary experiments on 16 specimens from Sahara showed relative visibility of achromatic zones to be better in the extracts of the liver, kidney, and brain, but zymograms of all eight tissues were qualitatively identical with those of black rockfish as described by Numachi (1972). Therefore in the second series of experiments on 44 specimens from Sahara and 94 from Kumaishi, only extracts of the liver and brain were used.

Fig. 1. Three electrophoretic patterns of tetrazolium oxidase in walleye, *Theragra chalcogramma*. Presumed subunit compositions of respective isozymes are shown on the underside.

Three phenotypes were detected in all tissue samples. These phenotypes are designated S (slow), F (fast) and SF. S and F phenotypes correspond to two single-bands of different mobilities, and the SF phenotype consisting of both of these single bands plus a band of intermediate mobility. The zymogram patterns agree with the suggestion (Baur & Schorr 1969; Utter 1971; Numachi 1972) that the TO have dimeric construction which is controlled by two codominant alleles (TO^s and TO^f) at the pollock TO locus.

As the statistical analysis reveals ($\chi^2=0.333$, d.f.=2) sexual dimorphism has no bearing on the gene frequencies of the TO alleles in walleye pollock. In the individual sample, the observed frequencies of phenotypes were adapted for expected frequencies assuming the Hardy-Weinberg equilibrium (Table 1). The levels of significance of the

Table 1. Observed and expected* frequencies of tetrazolium oxidase phenotypes in walleye pollock

Location	No. of fish	Phenotypes			Allelic frequency		
		S	SF	F	TO^s	TO^f	χ^2
Kumaishi	94	26 (24.5)	44 (46.9)	24 (22.6)	0.5106	0.4894	0.378
Sahara	60	12 (13.5)	33 (29.9)	15 (16.6)	0.4750	0.5250	0.634
Hachinohe	96	14 (11.3)	38 (43.3)	44 (41.4)	0.3438	0.6563	1.443

* Figures in parentheses assuming Hardy-Weinberg equilibrium.

Table 2. *Chi* square values and its probabilities analysed among each sample

χ^2 P	Kumaishi	Sahara	Hachinohe
Kumaishi	—	1.3674	7.8131
Sahara	0.75>P>0.5	—	6.8157
Hachinohe	0.025>P>0.01	0.05>P>0.025	

calculated *chi* square values for the samples of Kumaishi, Sahara and Hachinohe were $0.9 > P > 0.75$, $0.75 > P > 0.5$, and $0.5 > P > 0.25$ respectively. These probabilities show that observed frequencies of each allele fitted in with expected frequencies, that is, two TO alleles equilibrate in each fish group. Further, statistical difference of these frequencies was not detected among the samples of Kumaishi and Sahara, whereas significant differences were recognized between those of Hachinohe and Kumaishi or Sahara (Table 2). Obviously, further stock analysis is required to separate the walleye pollock populations on the basis of TO phenotypes.

The author wishes to express sincere gratitude to Dr. Ken-ichi Numachi of the Ocean Research Institute, the University of Tokyo for his valuable suggestions. Thanks are also due to Professor Ikusô Hamai, Associate Professor Takeshi Shibata, and Mr. Naoyuki Uchida for their kind guidance.

LITERATURE CITED

- Baur, E. W., and R. T. Schorr, 1969 Genetic polymorphism of tetrazolium oxidase in dogs. *Science* **166**: 1524-1525.
- Brewer, G. J., 1967 Achromatic regions of tetrazolium stained starch gels: Inherited electrophoretic variation. *Am. J. Hum. Genet.* **16**: 674-680.
- Johnson, A. G., F. M. Utter, and H. O. Hodgins, 1970 Interspecific variation of tetrazolium oxidase in *Sebastes* (Rockfish). *Comp. Biochem. Physiol.* **37**: 281-285.
- Numachi, K., 1972 Genetic polymorphism of tetrazolium oxidase in black rockfish. *Bull. Japan. Soc. Sci. Fish.* **38**: 789.
- Ridgway, G. J., S. W. Sherburne, and R. D. Lewis, 1970 Polymorphism in the esterases of Atlantic herring. *Trans. Amer. Fish. Soc.* **99**: 147-151.
- Utter, F. M., 1971 Tetrazolium oxidase phenotypes of rainbow trout (*Salmo gairdneri*) and Pacific salmon (*Oncoroychus* spp.). *Comp. Biochem. Physiol.* **39B**: 891-895.