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Isozyme Polymorphisms in Lingcod, *Ophiodon elongatus;* a Potential Tool for Stock Identification

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Isozyme polymorphisms in lingcod, Ophiodon elongatus; a potential tool for stock identification.

by

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

INTRODUCTION

Mortality, recruitment mechanisms and breeding units often operate at the population level of a species. Consequently, many fisheries programs attempt to define the populations, or stocks, when developing management strategies. Reproductively isolated populations tend to diverge genetically from one another. This divergence accumulates over time if isolation persists, and can often be measured by different frequencies of genetic variants. Biochemical genetic techniques are a particularly useful means for identifying genetic variants in a species and for measuring differences in the frequencies of these variants among conspecific populations (Allendorf and Utter 1979).

Lingcod, Ophiodon elongatus, support both sport and commercial fisheries in Washington State. In recent years catches have been declining, most notably in southern Puget Sound where a fishing moratorium has been in effect since 1978 (Ilg et al. 1979). Although stocks have not been identified, this fundamental information could be useful in developing future management plans. The purpose of this preliminary investigation was to assess the feasibility of using biochemical genetic techniques for stock identification.

MATERIALS AND METHODS

Tissue samples from lingcod for use in starch-gel electrophoresis were collected from several sources: the sport fishery at Neah Bay, WA; Grays Harbor, WA (Washington Department of Fisheries trawl survery for juveniles); and Newport, OR (Oregon Department of Fish and Wildlife). Samples were frozen as soon as possible after capture and transported to the Manchester Field Station (NMFS), Manchester, WA, where they were stored at $\leq -30^{\circ}C$ until processing.

Four tissues (muscle, eye, liver and heart) were screened in the isozyme survey. Starch-gel electrophoresis procedures followed those described by Utter et al. (1974), except that an agar overlay was used to stain the isozymes. Designations for starch-gel buffers are presented in Table 1. Twenty-four enzyme systems were examined (see Table 2 for abbreviations and corresponding enzymes).

RESULTS

Thirty-nine loci were hypothesized, based on the intensity and distribution of the zones of activity, three (PGI-2, ADH and ADA-1) of which (i.e. 8%) were polymorphic. Enzymes, tissues displaying the best activity, starch-gel buffers employed and the number of identified loci are presented in Table 2. A discussion of each of the polymorphic locus follows.

PGI (phosphoglucose isomerase) - Three phenotypes were observed for this system including one six banded and two three banded patterns (Figure 1). The relative mobilities and intensities of the banding patterns observed could best be interpreted as the result of two loci coding for a dimeric enzyme. Under this model both of the three banded phenotypes would reflect homozygous individuals for different allelic forms of the fast locus (AA & BB), and consist of two homodimer bands and an intermediate interlocus heterodimer. The six banded phenotype was presumed to reflect heterozygous individuals (AB); this phenotype expressed the sum of the bands of the 2 homozygous phenotypes plus an interlocus heterodimer band for the PGI-2 locus.

Gene frequencies were calculated under the above model for two locations, Neah Bay and Grays Harbor, WA, (Table 3); both groups were in Hardy-Weinberg equilibrium. The 95% confidence intervals between groups overlapped. A chi-square contingency

test between the groups was not significant, indicating that no gene frequency differences could be detected at this locus ($X^2 = 0.003$).

ADH (alcohol dehydrogenase) -The two single and one triple banded phenotypes observed for ADH (Figure 1) were assumed to reflect a dimeric enzyme coded for by a single disomic locus. Gene frequencies for Newport, OR did not depart significantly at the 95% level from Hardy-Weinberg proportions (Table 3).

ADA (adenosine deaminase) - Both heart and muscle tissue displayed a common zone of anodal activity. An invariant faster zone was observed in the heart only. Parallel expression of two phenotypes for the slow zone was observed in the two tissues. The common phenoytpe was represented by a single band, and the variant phenotype consisted of two bands of equal intensities with the slower band being the same mobility as the single banded phenotype. Therefore, a monomeric system coded for by one disomic locus was assummed. Gene frequencies were calculated for a small sample from Neah Bay, WA (Table 3).

Average Heterozygosity - The average heterozygosity ($\bar{H}$) of this species was estimated to be 0.022 according to the formula

$$H = (L - \sum_{i=1}^{L} \sum_{j=1}^{A_i} P^2_{ij}) / L$$

where L is the number of loci examined, A_i is the number of alleles at a given locus, and P_{ij} is the frequency of the j^{th} allele at the i^{th} locus. It is certainly possible that some of the zones we interpreted as monomorphic loci were actually interlocus heterodimers. Consequently, there may be fewer loci than we estimated. Therefore, our calculated heterozygosity may well be a conservative estimate.

CONCLUSIONS

Due to the limited quantity of available samples and difficulty with the proper frozen transport of others, allele frequencies of the three polymorphic loci could not be compared among all locations. Thus, an assessment of population structure based on these loci is not possible. However, information presented in this paper indicates that this line of research on lingcod could be rewarding.

The level of average heterozygosity (0.022) and proportion of polymorphic loci (8%) for lingcod is at the low end of the spectrum relative to surveys made over wide taxonomic ranges of organisms (e.g. Selander, 1976). Nevertheless, these values lie within ranges reported for other carnivorous marine fishes (e.g. $\bar{H}$ range in Sebastes sp. 0.004-0.06, Wishard et al., 1980). In walleye pollock, Theragra chalcogramma, 2 of 28 loci (7%) had variants in great enough frequencies to be useful in distinguishing populations (Grant and Utter 1980); lingcod are suitably polymorphic at 8% of the loci examined. Based on these comparisons, this initial study suggests that the amount of genetic variation observed in lingcod could be useful in stock identification and warrants further investigation. Any future programs directed toward defining populations utilizing biochemical genetic analyses should endeavor to obtain sufficiently large samples (n = 50-100) over the expanse of the species range; Kodiak, Alaska to Pt. San Carlos, Baja, California (Miller and Geibel 1973). Additionally, oceanographic

pockets which may potentially be isolated from the coastal habitat, i.e. south Puget Sound and the Strait of Georgia should be included. To acquire the greatest amount of information pertaining to population structure biochemical genetic analyses should be executed in conjunction with other more classical analyses such as morphological, fecundity and growth investigations.

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Abbre-viation	Major Components of Buffers	pH	Author
TRI	Triethanolamine citrate	8.0	Clayton and Tretiak (1972)
AC	N-(3aminopropyl)-morpholine citrate	6.5	Clayton and Tretiak (1972)
MF	Tris-boric acid- EDTA	8.5	Markert and Faulhaber (1965)
TME	Tris-malate-EDTA	7.4	Harris and Hopkinson (1976)
RW	gel: Tris-citric acid	8.5	Ridgway et al. (1970)
	electrode: Lithium hydroxide-boric acid	8.1	

Table 1. Buffer systems used in starch-gel electrophoresis.

Abbreviation	Enzyme	Tissue[1/]	Starch-gel buffer	Number of loci: polymorphic	Number of loci: monomorphic	Comments
AAT	Asparate amino transferase	H,L	TME	0	2	Cathodal zone may be polymorphic; weak activity.
ACP	Acid phosphatase	H	AC,TRI	0	1	
ADA	Adenosine deaminase	M,H	MF	1	1	The faster, monomorphic locus appeared only in heart.
ADH	Alcohol dehydrogenase	L,	AC	1	0	Cathodal.
AGP	Alphaglycero-phosphate dehydrogenase	L,M,H	TME	0	2	Fast locus appeared only in heart.
EST	Esterase	H	TRI	-	-	Uninterpretable.
FUM	Fumerase	-	-	-	-	Very weak activity in all tissues.
GDA	Guanine deaminase	L,H	MF	0	1	
GP	General protein	M	RW	0	1	
IDH	Isocitrate dehydrogenase	H,M,E,L	TME	0	3	The fastest locus appeared only in liver.
LDH	Lactate dehydrogenase	H	RW	0	2	
MDH	Malate dehydrogenase	H,L	AC,TME	0	3	
ME	Malic enzyme	H,L,M	AC,TME	0	3	Appears the same as MDH.
PEP	Peptides					
AL-TY	Alanyl-tyrosine dipeptide	H,L,M	TME	0	1	
GL	Glycyl-leucine dipeptide	H,L,M	TME,TRI	0	1	
LGG	Leucyl-glycyl-glycine tripeptide	H,L,M,E	AC,MF	0	3	The third locus had the same mobility as PHAP-2.
PHAP	Phenyl-alanyl-proline tripeptide	H,L,M	TME	0	2	
6PG	6-phosphogluconate dehydrogenase	L,H	TRI	0	2	The slower locus occurred only in heart.
PGI	Phosphoglucose isomerase	H,L	TME,RW	1	1	Fast locus is polymorphic (PGI-2).
PGM	Phosphoglucomutase	H,L	TME	0	3	
PMI	Phosphomannose isomerase	H,M,E,L	AC,TME	0	2	Only slow locus occurred in liver.
SDH	Sorbitol dehydrogenase	-	-	-	-	No activity.
TO	Tetrazolium oxidase	H,L	TME	0	1	
XDH	Xanthine dehydrogenase	H,L,E,M	TRI	0	1	
		Total number of loci:		3	36	

1/ Tissue designations: E = eye, H = heart, L = liver, M = muscle.

Table 2. Enzymes surveyed for the lingcod, Ophidon elongatus. Tissues and starch-gel buffer listed are those which yielded the best activity and resolution. The number of polymorphic and monomorphic loci for each enzyme are presented.

Location	Date	Sample Size	Number of phenotypes AA	AB	BB	Gene frequencies a	b	X^2
			P G I					
Neah Bay, WA	May 1979	47	29	16	2	.79	.21	.024
Gray's Harbor, WA	July 1980	60	43	14	3	.83	.17	1.52
			A D H					
Newport, OR	August 1980	48	22	21	5	.68	.32	.01
			A D A					
Neah Bay, WA	July 1979	9	8	1	-	.94	.06	-

Table 3. Gene frequencies in polymorphic loci (PGI, ADH, AFA) for samples collected at various locations in Washington State.

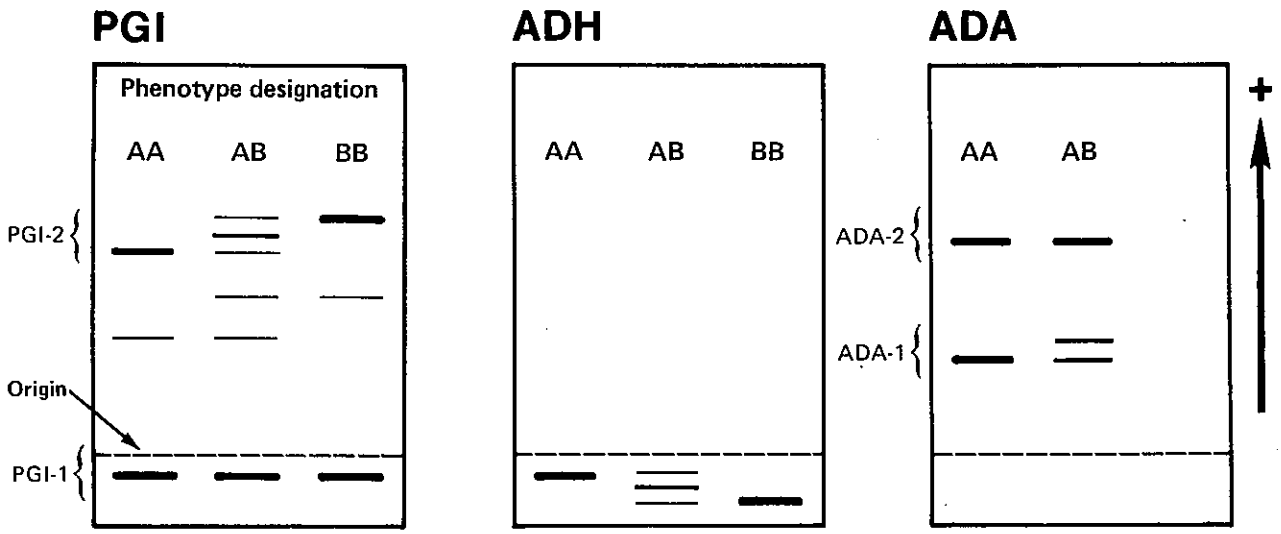

Figure 1. Electrophoretic patterns observed for PGI, ADH, and ADA. Banding patterns observed between PGI-1 and PGI-2 were interpreted as interlocus heterodimers.