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Preliminary Results of Biochemical-genetic Population Structure Study of the Squid *Illex illecebrosus*

by

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Introduction

It is still uncertain as to whether the total distribution of I. illecebrosus constitutes a single identifiable stock or a number of discrete populations. Current thoughts on life cycle suggest that a single breeding stock forms the summer concentrations found on the Scotian shelf and Eastward. On the other hand Georges Bank and Southward, may have two cohorts that probably results from two spawning periods. To further understand unit stock and population structure, this biochemical-genetic study was undertaken.

Gel electrophoresis was used in an attempt to identify polymorphic enzymes that may distinguish between genetically different populations of Illex. Ally and Keck (1978) and Christofferson et al. (1978) have shown that phosphoglucomutase (PGM) and glutamate oxaloacetate transaminase (GOT) are sufficiently polymorphic for this purpose in a similar study of the squid, Loligo opatescens. It is the purpose of this study to identify and then use a polymorphic enzyme as marker to determine the degree of heterogeneity between many samples collected along the Northwest Atlantic.

Materials and Methods

The squid analysed to date come from the Scotian Shelf and were obtained during the June cruise of the Lady Hammond at the

following locations: station 007 at 40° 31'N, 65°06'W on June 2; station 022 at 44°22'N, 57°35'W on June 5; station 032 at 44°04'N, 58°54'W on June 6, and station 048 at 43°59'N, 63°37'W on June 7.

The Illex were collected at a depth of 110 - 152 m, and immediately after capture standard morphometric analyses (Amaratunga and Durward, 1979) were conducted. A sample of mantle (approximately 20 grams) and liver was collected from each animal and frozen individually at -25°C.

On arrival at the laboratory the samples were homogenized in distilled water with mortar and pestle. The homogenate was then centrifuged at 10,000 RPM for 20 minutes.

The samples were tested in five buffer systems; Tris-citrate (TC) (Siciliano and Shaw, 1976), and the specific buffer and staining systems for esterase (Est), leucine aminopeptidase (Lap) octanol dehydrogenase (Odh), aldehyde oxidase (Ao), xanthine dehydrogenase (Xdh), glucose-6-phosphate dehydrogenase (G-6-Pd), 6-phosphogluconate dehydrogenase (6-Pgd), malic enzyme (ME), phosphoglucomutase (Pgm), α-glycerophosphate dehydrogenase (α-GPD), malate dehydrogenase (Mdh), and alkaline phosphatase (Aph) all prepared according to Loukas and Krimbas (1980).

A 12% starch-gel electrophoresis medium was prepared according to Smithies (1955, cited by Ally and Keck, 1978). The buffer system for the preparation of the starch gel varied among enzymes as shown in Table 1. Filter paper applicators were inoculated with the enzyme extract and placed into a slit cut into the gel. Horizontal electrophoresis was carried out at 10°C for the time and at the constant current required for each buffer system. The gel was then stained using the required histochemical mixture. The gels were fixed in a 5:5:1 solution of water methanol acetate acid. The quality of the gels was recorded as follows: no activity; poor, for diffuse indistinguishable bands; and fair to very good for increasing sharpness and clarity of the bands.

Results and Discussion

Table 1 gives the tissue in which each enzyme is most active and the number of animals tested from each sample. The quality of the gel and whether a polymorphism was observed in the gel is also shown for each system.

The enzymes PGM, Got, acid phosphatase (AP) and phosphoglucose isomerase (PGI) were selected as possible markers for the comparison of samples. The mantle tissue alone was used because it gave consistently good bands for most enzymes.

Table 2 provides the frequency of polymorphic enzymes in each sample (data for sample 022 was not complete at time of this presentation).

The results indicate that populations of Illex from the Scotian Shelf contain little genetic variability. The low frequency of polymorphic enzymes may be characteristic of this area alone or may be indicative of all areas along the Northwest Atlantic. Since samples from distinct locations have not yet been tested, we cannot conclude the possibility that the electrophoretic differences between such populations will be found. The observation that squid schools are genetically monomorphic is by itself of some interest given that most populations of sexually reproducing species contain usually larger levels of variability.

References

Ally, J.R. Raymond and Scott A. Keck. 1978. A biochemical-genetic population structure study of market squid, Loligo opalescens, along the California Coast. Calif. Fish. Bull. 169: 113-121.

Amaratunga, T. and R. D. Durward. 1979. Standaridzation of data collection for the short-finned squid, Illex illecebrosus. ICNAF Res. Doc. 78/II/5.

Christofferson, J.P., A. Foss, W. E. Lambert and B. Welge. 1978. An electrophoretic study of select proteins from the market squid, Loligo opalescens, Berry. Calif.Fish Bull. 169: 123-133.

Loukas, M. and C.B. Krimbas. 1980. Isozyme techniques in D. subobscura. D.I.S. 55: 157-158.

Siciliano, M.J., and C. R. Shaw. 1976. Separation and visulaization of enzymes on gels. P. 185-209. In: I. Smith (Ed.) Chromatographic and electophoretic techniques, Vol. 2. William Heinemann Medical Books Ltd., London.

Table 1

Enzyme	Buffer System	Tissue	Location (# animals)	Comments A Quality of Gels	Comments B Presence or Absence of Polymorphism
AP-1	Tris-Citrate	Mantle and Liver	007 (30) 032 (30) 048 (30)	Good	Absence
AP-2	"	" " "	" "	Good	Possible
GOT I	Tris-Citrate	Mantle	007 (30) 032 (30) 048 (30)	Good	Presence
GOT II	"	Liver	" "	Good	Presence
PGI	Tris-Citrate	Mantle	007 (30) 032 (30) 048 (30)	Good	Presence
PGM	Tris-Citrate	Mantle	007 (30) 032 (30) 048 (30)	Fair	Presence
GP	Tris-Citrate	Mantle & Liver	007 (30) 032 (30) 048 (30)	Fair	Possible
AO	Tris	Liver	048 (10) 007 (10)	Good	Absence
G-6-PD	Tris	Liver	048 (10) 007 (10)	Fair	Absence
Xdh	Tris	-----	048 (10) 007 (10)	No activity	-----
6-PGD	Tris	-----	048 (20) 022 (10)	No activity	-----
Aph	Tris-citric Boric-LiOH	Liver	048 (20) 022 (10)	Good	Absence
LAP	"	"	048 (20) 022 (10)	Good	Possible
Est	"	Liver and Mantle	048 (20) 022 (10)	Good	Absence
ME	Tris-Malic acid-EDTA, $MgCl_2$	Mantle and Liver	007 (20)	Fair	Absence
PGM	"	Mantle and Liver	007 (20)	Poor	Could not be read
T.O.	"	Mantle and Liver	007 (20)	Good	Absence
α - GPD	"	-----	007 (20)	No activity	-----
Mdh	"	-----	007 (20)	No activity	-----
PGM	Tris-Versene Borate	Mantle	007 (20) 048 (20)	Poor	Not readable
GOT	"	"	007 (20) 048 (20)	Poor	Not readable

Table 2

The frequency of polymorphism in Each Enzyme

Station	PGM	GOT	AP	PGI
048	1/42	0	0	0
032	0	1/50	1/50	0
007	2/50	0	0	0
022	1/14	0	0	0