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ELECTROPHORETIC STUDY ON MUSCLE AND EYE LENS PROTEINS OF THREE SCIAENIDS

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ABSTRACT

Results of the electrophoretic study on muscle and eye lens proteins of three species of scianeids *viz. Johnius macrorhynus* (Mohan), *Johnieops vogleri* (Bleeker) and *Otolithes cuvieri* (Trewavas) is reported in the present communication. The number of protein fractions in the muscle of *J. vogleri, J. macrorhynus* and *O. cuvieri* were found to be 10, 11 and 14 respectively while the same for eye lens proteins as revealed by the densitometric scan were 10 for *J. vogleri* and 6 each for *J. macrorhynus* and *O. cuvieri* respectively.

The staining pattern of the muscle proteins shows that it was more intense for fraction 4, 8, 10 and 11 for *J. vogleri*, 6, 7, 9, and 10 for *J. macrorhynus* and 1, 3, 11 and 13 for *O. cuvieri*. In case of eye lens proteins the staining was more intense for fractions, 1, 3, 7, and 8 for *J. vogleri*, 1, 4, 5 and 6, for *J. macrorhynus* and 1, 2, 4 and 6 for *O. cuvieri*. The differences in the number of protein fractions, their mobility pattern and staining intensity indicates species specificity.

INTRODUCTION

In recent years the major biological tool applied in taxonomy of fish is electrophoresis. Study on the protein patterns have become a valuable tool in the elucidiation of taxonomic problems. In marine and fresh water fishes the proteins which have been most extensively studied include haemoglobins (Sick, 1961), erum plasma proteins (Nyman 1965 a,b) muscle myogens (Tsuyuki and Roberts, 1965, 1966; Tsuyuki *et al.*, 1965) and organ proteins (Nyman, 1965 b). The major features of the patterns, of such bands on the gel medium, have been shown to be species specific. It has also been possible to identify hybrid individuals between species of which the parent patterns are known and to recognize distinct population of the same species in different parts of the geographical range by minor differences in the patterns.

The present investigation was undertaken to compare, under uniform conditions the electrophoretic components of muscle and eye lens proteins of three species of sciaenids *viz. J. vogleri, J. macrorhynus* and *O. cuvieri* with regard to their mobility, amount and number of bands so as to determine the electrophoretic pattern characteristic of each species. Of these three species, *O. cuvieri* is found in the coasts of India and Pakistan, *J. macrorhynus* on the coasts of India, Srilanka, Andaman Islands and Singapore and *J. vogleri* on the coasts of Gulf of Oman, India, Indo-Australian Archipelago, Australia, Philippines, Thailand, S. China, Japan and east coast of Africa from Zanzibar to Natal (Day, 1976; Weber and de Beaufort, 1936 and Fisher and Bianchi, 1984).

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Material and Methods

Fresh samples were collected from the landing centre. They were packed in ice and brought to the laboratory. Protein extracts of muscle were made by taking 100 mg of dorsolateral muscle avoiding red muscles. This was mechanically homogenized in isotonic solution and after diluting ten times in isotonic solution was subjected to centrifugation at 4000 r. p. m. for 30 minutes. The eye lenses were removed from the fish and freed from aqueous and vitreous humeur, pieces of retina and capsule. Protein extract of lenses were made by homogenizing them in 0.9% saline solution. This was subjected to centrifugation at 5,000 r. p. m. for 30 minutes. Electrophoresis was carried out using polyacrylamide gel electrophoresis (PAGE) method of Davis and Ornstein (1961).

The running gel solution was prepared by mixing small pore buffer, monomer, double distilled water and ammonium per-sulphate in 1 : 2 : 1 : 4 ratio and was poured in the gel tubes. It was allowed to polymerize. The upper (pacer) gel was prepared by mixing large pore buffer, monomer solution, riboflavin and sucrose in the ratio of 1 : 2 : 1 : 4. Over the running gel solution in the tube, 0.15 ml of spacer gel solution was poured. Acrylamide of 7 and 5% were used for muscle and eye lens proteins repectively. In each tube 0.01 ml solution of the homogenized protein extract was added and electrophoresis was carried out at a constant current of 4 μA/ tube. A drop of amido black was added on the top of the gel tube as a tracing dye and when the same reached at the bottom of the tube, the power supply was switched off. The gels were stained overnight in 1% amido black prepared in 7% glacial acetic acid.

The intensity and position of different components were studied using photoelectric densitometer by putting a green filter at 540 mm. The electrophoretic fractions have been assigned numbers in order of increasing mobility from the point of application. The number of crest corresponds to the number of distinct proteins and the area under the crest is proportional to their concentration (Carpenter, 1965). The relative proportion of each fraction was estimated using the following formula.

$$\text{Relative proportion (in\%)} = \frac{\text{No. of Squares occupied by part of the fraction}}{\text{Total No. of squares occupied by the whole scan}} \times 100$$

The study is based on 15 specimens of *J. macrorhynus* and 12 specimen each of *J. vogleri* and *O. cuvieri*.

Results

The pattern of densitometric scan of the protein patterns separated in the gel is presented in Figs. 1 and 2 for muscle and eye lens proteins respectively. The number of protein fractions in the muscle of *J. vogleri*, *J. macrorhynus* and *O. cuvieri* were found to be 11, 10 and 14 respectively. The distance travelled by each fraction from the point of application is given Table 1. Each protein fraction indicated species specificity showing differences in the mobility pattern. The differences between the species in the final mobility was also observed - the last protein fraction travelling upto 92 mm in *J. vogleri*, 86 mm in *J. macrorhynus* and 84 mm in *O. cuvieri*.

The relative mobility in of protein fraction in percentage for muscle proteins is given in Table 3. It revealed that staining was more intense for fraction 4, 8, 10 and 11 for *J. vogleri*, 6, 7, 9, and 10 for *J. macrorhynus* and 1, 3, 11 and 13 for *O. cuvieri*.

The number of protein fractions in the

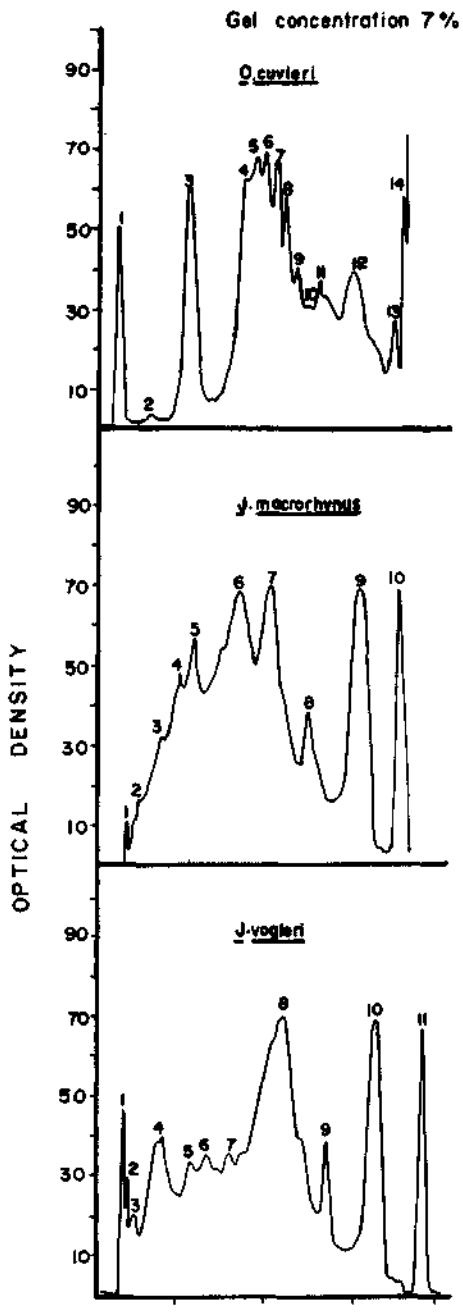

Fig. 1. The scanning pattern of the electropherograms of muscle proteins of three species of sciaenids.

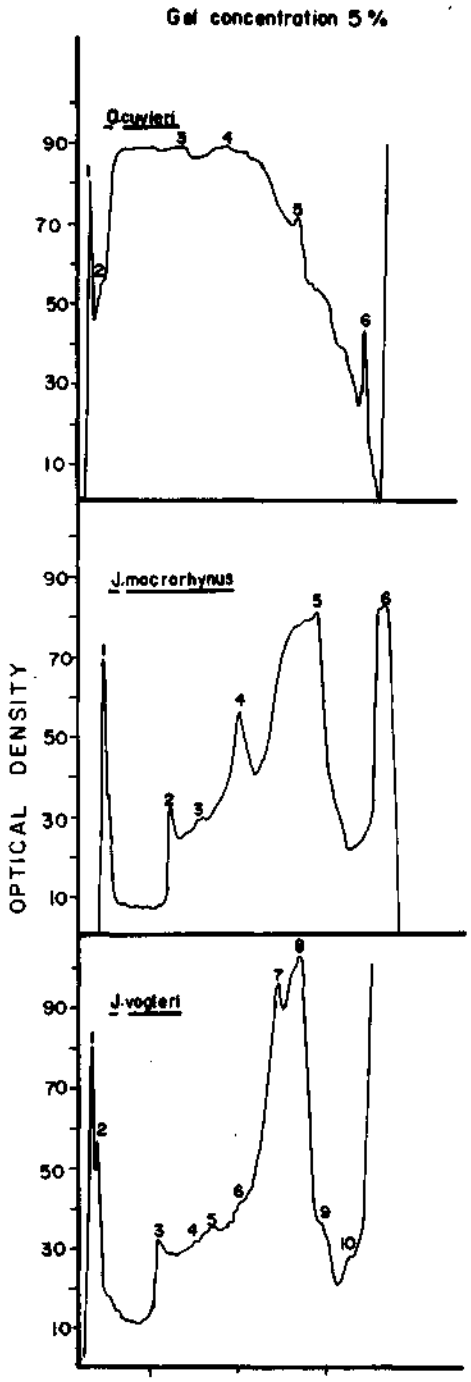

Fig. 2. Scanning pattern of electropherograms of eye lens proteins of three species of sciaenids.

eye lens were found to be 10 for *J. vogleri* and 6 each for *J. macrorhynus* and *O. cuvieri* respectively. The mobility pattern of the same is presented in Table 2. Here too each and every protein fraction in all the three species differed in their mobility patterns. The mobility of last fraction was found to be 78, 87 and 79 mm for *J. vogleri, J. macrorhynus* and *O. cuvieri* respectively.

The relative mobility in percentage of the protein fraction for eye lens proteins is given in Table 4. The staining was intense for protein fractions 1, 3, 7 and 8 for *J. vogleri*, 1, 4, 5 and 6 for *J. macrorhynus* and 1, 2, 4 and 6 for *O. cuvieri*.

Discussion

Tsuyuki *et al.* (1965) have discussed the value of muscle myogen patterns in the phylogenetic studies and inter and intra specific protein variation as diagnostic characteristics of stock analysis. Earlier the only method available for the study of interspecific and racial variation was the morphometric and meristic characters. However, at present biochemical genetics has become an useful tool to supplement the same.

Table 1. *Distance moved in mm of different muscle protein fractions from the point of sample application*

Species	Fractions (in order of slowest to fastest)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
J. vogleri	6	7.5	10	17.5	26	31	37.5	52	64	78	92	—	—	—
J. macrorhynus	8.5	11.5	18	23	27	41	49	60	75	86	—	—	—	—
O. cuvieri	4.5	13	25	41	44.5	47	51	53	56	60	62	72	83	87

Table 2. *Distance moved in mm of different eye lens protein fractions from the point of sample application.*

Species	Fractions (in order of slowest to fastest)									
	1	2	3	4	5	6	7	8	9	10
J. vogleri	4	5.5	23	33	39	46	57	63	70	78
J. macrorhynus	7	26	33	46	69	87	—	—	—	—
O. cuvieri	3.5	8	32	43	64	79	—	—	—	—

Table 3. *Relative mobility of fractions in percentage for muscle proteins*

Species	Fractions													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
J. vogleri	0.98	0.38	0.72	7.45	0.97	0.97	0.64	30.84	3.44	27.81	25.80	—	—	—
J. macrorhynus	0.97	0.94	0.93	0.47	3.27	13.08	12.15	4.20	44.39	19.62	—	—	—	—
O. cuvieri	24.90	1.59	46.03	1.05	1.05	1.59	2.64	2.11	0.53	0.53	3.17	8.52	5.0	1.29

TABLE 4. *Relative mobility of fractions in percentage for eye lens proteins*

Species	Fractions									
	1	2	3	4	5	6	7	8	9	10
J. vogleri	64.10	1.20	3.42	1.23	2.40	2.47	6.07	14.04	1.23	3.84
J. macrorhynus	10.55	1.39	2.22	5.00	52.5	28.34	—	—	—	—
O. cuvieri	30.76	15.38	7.69	15.39	5.13	25.65	—	—	—	—

Menezes (1976) studied the eye lens and serum proteins of *Sardinella longiceps* and *S. fimbriata*. She found that the eye lens proteins of the two species did not show marked differences in the number mobility or staining intensity of protein fractions. However, the comparative study of serum proteins of the two species revealed major differences in the protein fractions, mobility and amount of protein in various fractions which are characteristic of the particular species studied. Investigation on eye lens proteins of three species of flat fishes *Psettodus erumei, Brachiurus orientalis* and *Pseudorhombus arsius* by Menezes (1979) indicated significant differences in the number of protein fractions, their migratory distance and staining intensity. All these indicated a pattern which is characteristic of the species.

In the present investigation involving muscle and eye lens proteins of three species of sciaenids clearly indicate the electrophoretic patterns which are characteristic of the species. A comparison of the electrophoretic pattern, revealed that, apart from difference in the number and mobility pattern of each and every protein fraction, staining intensity also differed. Among the muscle proteins, fractions 4, 8, 10 and 11 for *J. vogleri*, 6, 7, 9 and 10 for *J. macrorhynus* and 1, 3, 11 and 13 for *O. cuvieri* shows intense staining. In the protein pattern of eye lenses, fractions 1, 3, 7 and 8 in *J. vogleri*, 1, 4, 5 and 6 in *J. macrorhynus* and 1, 2, 4, and 6 in *O. cuvieri* were intensely stained. The possibility that denaturation rather than genotype accounting for the variability is unlikely because the muscle and eye lenses were examined in fresh condition. Further, denatured tissues produce an electrophoretic pattern in which the lesser migrating fraction becomes more mobile to the extent of even fusing with the farthest migrating fraction so that only one pattern is observed (Sibby and Brush, 1967). Such type of mobility of fraction was not observed for any of the three species studied here.

As all the three species of sciaenids have wide distribution, the electrophoretic pattern thus obtained would be very useful to compare with the electrophoretic patterns of these species occuring in other parts of the world. The minor differences in the electrophoretic patterns would help us recognise distinct geographic populations.

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