

# INTRASPECIFIC VARIATION IN THE SOLUBLE NUCLEAR EYE LENS PROTEINS OF THE MULLET, *MUGIL CEPHALUS* (L.)

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## ABSTRACT

The technique of electrophoretic separation of nuclear eye lens protein was applied for studying genetically based molecular variations in the mullet *Mugil cephalus* L. The nuclear eye lens protein was extracted using 0.0189% NaCl solution. Electrophorograms produced five pattern types reflecting heterogeneity. Some variations were found to be due to the size of the fish and therefore their ontogenetic basis is discussed.

## INTRODUCTION

Soluble proteins of the nuclear region of a vertebrate eye lens provide a unique molecular system for studying genetically based differences between animals (Smith, 1962, 1966a, 1969b; Smith and Goldstain, 1967). Protein in the nucleus of the eye lens is an ideal material for genetic studies because of ease in the collection and storage of lenses, stability against seasonal environmental and metabolic changes (Smith, 1969b; Smith and Goldstain, 1967; Peterson and Smith, 1969).

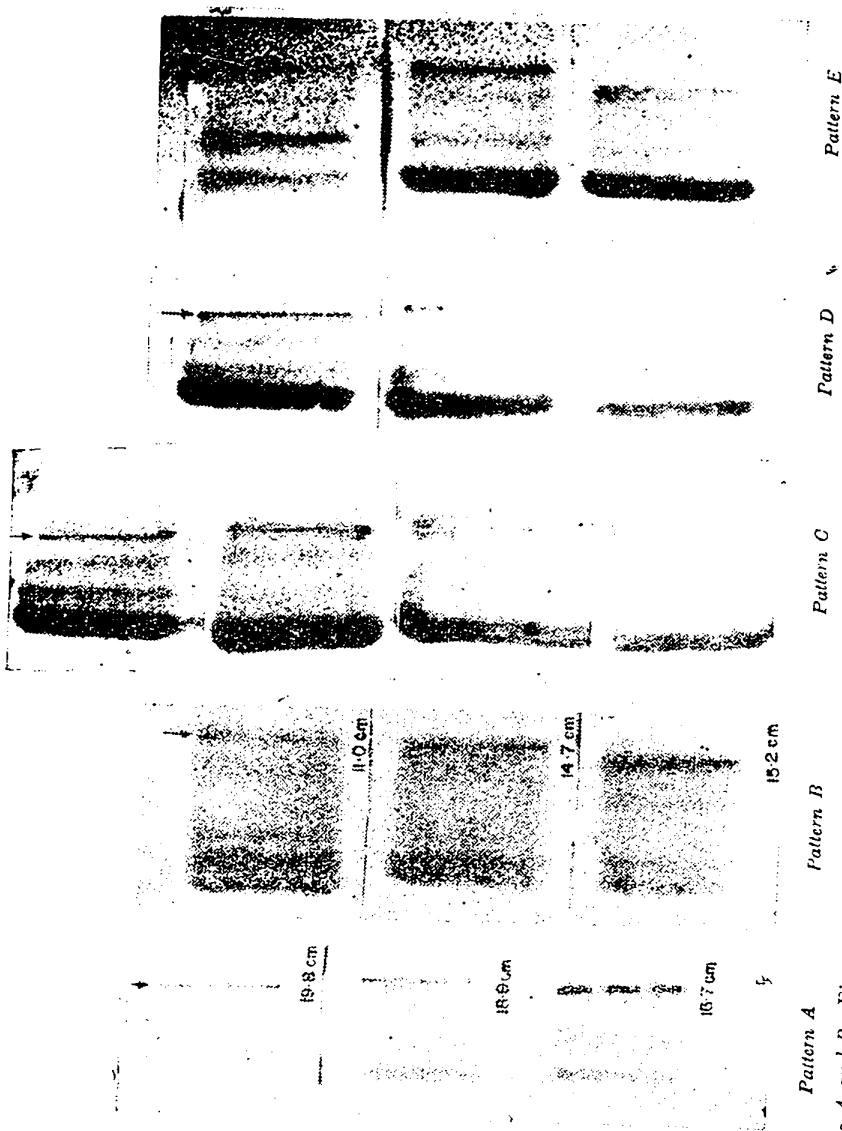
Genetically based intraspecific variations in nuclear eye lens protein have been reported earlier (Smith, 1966b, 1971b, 1971c; Eckroat and Wright, 1969, Peterson and Shehadeh, 1971) and these have been used to identify separate populations of the same species. However, some variations are believed to be due to ontogeny (Peterson and Shehadeh, 1971).

In the present study, the electrophoretic method was applied to determine if heterogeneity existed in the nuclear eye-lens proteins of mullet *Mugil cephalus* and if so, to what extent.

## MATERIAL AND METHOD

Eye lenses were collected from deep frozen mullets, *Mugil cephalus*. Lenses were removed, weighed and macerated in a mortar, and 0.0189% saline solution equal to seven times the weight of the nuclear tissue was added. This particular saline solution is especially advantageous because it is strong enough to extract both the major classes of proteins such as albumins and globulins and is too weak to cause undesirable "salt effects" in the electrophoretic patterns (Smith 1968; 1970).

The extracts were cleared by centrifugation for five minutes at 3000 rpm and then processed as described earlier (Bhosle, 1976). After electrophoresis, the cellulose acetate strips were soaked consecutively for five minutes in each of the three solutions. Ponceau S (0.4 gm) in 5% trichloroacetic acid (100 ml), 5% glacial acetic acid and absolute methanol. After the methanol rinse, they were immersed for a few seconds in a solution containing 80 parts of absolute methanol and 20 parts of 5% glacial acetic acid. Each strip was then dried on a glass plate.



*Patterns A and B:* Electrophoretic pattern of nuclear eye lens proteins of mullet of different size as measured by standard length. Note the relative decrease in fraction I (Pattern A) and increase (Pattern B). Arrow indicate the point of sample application.

*Pattern C:* Electrophoretic pattern of mullet nuclear lens proteins which migrated towards the cathode and anode. Arrow indicate same as in Patterns A and B.

*Patterns D and E:* Electrophoretic patterns of mullet nuclear lens proteins which migrated only towards cathode (Pattern D) and toward cathode and anode (Pattern E). Arrow indicate same as in patterns A-C.

## RESULTS

Electrophoretic separation of the nuclear eye lens proteins of *Mugil cephalus* produced five different patterns. The pattern A (see Fig.) indicates four proteins, all on the cathodal side. In this pattern, fraction 1 stains very lightly. Pattern B is composed of four proteins of which three appear to be similar to those found in pattern A. The fraction 1 is relatively broad and intensely stained in pattern B. Pattern C produced five proteins of which four were similar to patterns A and B whereas the fifth protein is on the anodal side and gets very lightly stained. Pattern D consists of three proteins of which two are moderately stained, whereas the third protein is broad and takes dark stain. Pattern E has four proteins of which three are similar to pattern D and one very lightly stained which moves towards the anode.

## DISCUSSION

Results obtained from the electrophoretic separation of nuclear lens showed two main types of variations: (i) those which are co-related with the size of the fish (Patterns A-C), (ii) those which were independent of the size (Patterns C-E). The graded nature of the variation in fraction 1 of the mullet suggests a direct relationship with ontogeny (Peterson and Shehadeh, 1971). Ontogenetic basis was suggested by Barrett and Williams (1967), while studying the whole lens proteins of bonito, *Sarda chiliensis* using starch gel electrophoresis. This interpretation was not supported either by Eckroat and Wright (1969) or Smith (1971b) who also studied the bonito eye lens proteins using cellulose acetate electrophoresis. However, different components of proteins are found between embryonic and adult

lenses (Francois and Rabaey, 1957; Papaconstantinou, 1965; Mehta and Maisel, 1968; Rana and Maisel, 1969) and between young and old lenses (Cobb and Koenig, 1968) in several animals and this appears to suggest an ontological explanation for the patterns A and B.

A general decrease in the staining intensity of the nuclear eye lens proteins of older mullets agrees with the findings that in the vertebrate lens, the concentration of soluble protein progressively decreases due to its conversion into insoluble protein like albuminoid (Dische, Borenfreund and Zelmenis, 1956; Lerman, Tuttle and Koser, 1968; Manski, Behrens and Martinez, 1968) and due to decrease in the synthesis of soluble crystallins (Dische, Borenfreund and Zelmenis, 1956; Lerman and Fontaine, 1962; Peterson and Smith, 1969; Peterson and Shehadeh, 1971).

Electrophoretic pattern types shown in patterns C-E indicate genetic polymorphism. Seven samples of mullet showed the presence of lightly stained protein bands which migrated a short distance towards the anode and, therefore, these are acidic. Polymorphic acidic proteins were previously described in bluefin tuna (Smith, 1966a, b), ocean white fish (Smith and Goldstein, 1967), mackerel scad (Smith, 1969b). The acidic protein was present only in a few samples. Most of the patterns obtained had negligible amounts of this protein. This type of variation probably has a genetic basis (Smith, 1971c).

The absence of a cathodal protein in the twenty samples analysed in the present investigation suggests the existence of basic protein polymorphism. The phenomenon of polymorphism of highly basic protein has been described in bonito

(Smith, 1971b) and sandbar shark (Peterson, 1970; Peterson and Smith, 1969).

The possibility that the variation in the protein composition of eye lens nucleus is due to denaturation rather than genotype seems unlikely because nuclear protein strongly resists breakdown (Smith, 1965, 1971; Bhosle, 1976).

The preliminary data reveal heterogeneity of nuclear eye lens protein and thus support the earlier findings that

protein variability within the species can be used to identify separate breeding populations.

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