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ELECTROPHORETIC STUDIES ON THE SERUM PROTEINS OF THE THREE SPECIES OF GENUS *CHANNA*

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ABSTRACT

The blood serum proteins of the three species of fish belonging to the Genus *Channa* were examined by polyacrylamide 'disc' electrophoresis. Although 10 main fractions were resolved in all the three species, each species showed a characteristic pattern which is highly reproducible and the differences are significant.

Study of the genetic variants of proteins has been in the past successfully applied to diverse fishery problems and hybrid verification (Aspinwall & Tsuyuki, 1963). Genetic markers associated with characters of commercial importance such as growth and percentage hatch would be of obvious economic value for fish culturists.

Although a good number of studies have been conducted on the fundamental analysis of fish sera components to identify them and also to note genetic polymorphism of some protein fractions, (Hattingh, 1974; Jimenez & Planas, 1973; Perrier *et al.*, 1973; 1974, 1977; Harris, 1974), little information is available on Indian fishes. Therefore a study of the serum proteins of three species of an economically important edible fish belonging to Genus *Channa* viz *C. punctata*, *C. striata* and *C. gachua* was conducted.

The study was aimed at identifying the serum proteins which can form a firm basis for further studies on genetic and biochemical variation, for breeding experiments utilizing polymorphic proteins as genetic markers in developments of better species, identification of sub-populations and other fishery problems.

The fishes used in this study were collected during their prespawning period and were all healthy and mature. Blood was collected from the severed caudal veins and artery after amputation of the peduncle of the fish into small glass tubes. The blood was allowed to clot and was then centrifuged at 3000 rpm for 10-15 minutes and the serum separated and used.

The proteins were analysed by disc electrophoresis with Tris-glycine buffer pH 8.1 (Clarke, 1964). The bands were stained with 0.5% Amido black 10B for proteins, periodic acid Schiff reagent for glycoproteins (Clarke, 1964), and Sudan black for lipoproteins (Perrier *et al.*, 1974). The method of Muller *et al.* (1962) was used to detect the iron bound to the transferrins. The ceruloplasmins and haptoglobins were visualised using the staining procedure of Mc Combes (1969). The serum samples and Bowman were subjected to different temperatures ranging from 40 to 65°C to test the heat sensitivities of the proteins which were also precipitated using different concentrations of ammonium sulphate.

The electrophoretic patterns of sera of these three species of fish revealed distinct

bands. The protein fractions resolved in each case were numbered sequentially following decreasing order of mobility i.e. the most rapidly migrating being No. 1. The numbering was for the sake of convenience and does not imply homology of the like numbered fractions in the different species. In each species the serum proteins were separated on the same electrophoretic run, of which one gel was stained for the protein and the other for the specific component in question. By this it was possible to make direct comparison of the protein and other zones and hence to locate the iron, lipid, carbohydrate, haptoglobin and ceruloplasmin in terms of the protein zones.

In *C. punctata* as many as 17 fractions were resolved of which 10 was the maximum number that appeared with consistency (Fig. 1 & 4p). These fractions were numbered 1, 2 (a, b, c, d), 3 (a, b, c, d), 4, 5 (a, b), 6, 7, 8, 9 & 10. In *C. striata* (Fig. 2 & 4S) and *C. gachua* (Fig. 3 & 4G) about 10 to 14 protein zones could be distinguished in the normal fish sera, although in both species

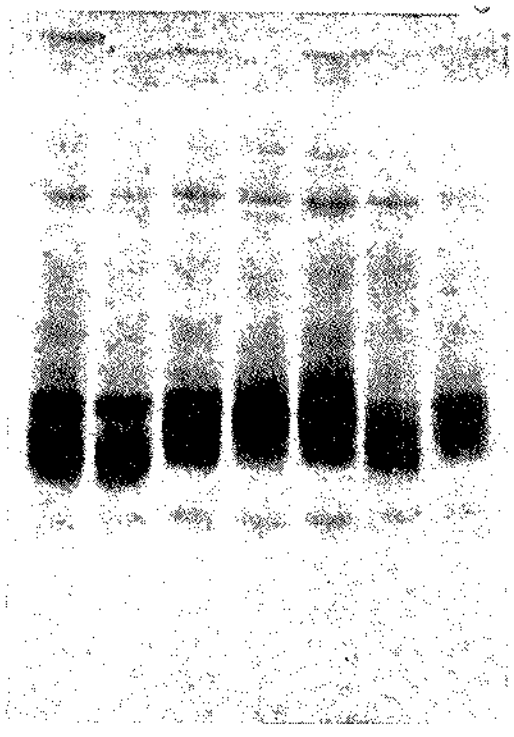

Fig. 1. Electrophoretic protein pattern of the serum of *C. punctata*.

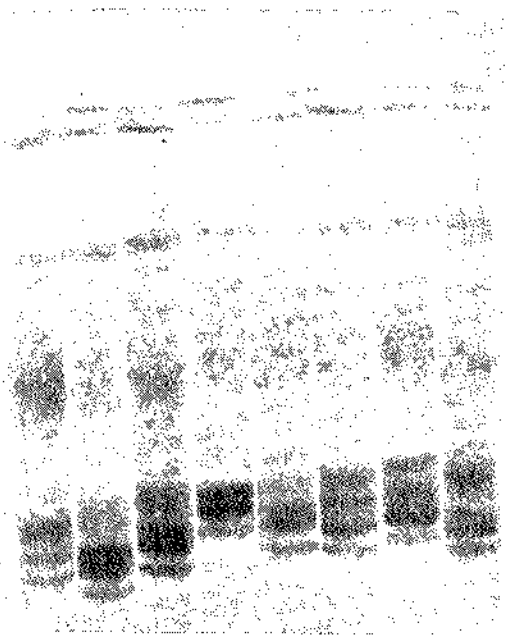

Fig. 2. Electrophoretic protein pattern of the serum of *C. striata*.

10 was the maximum number that appeared with consistency. The protein fractions were numbered 1, 2 (a, b, c, d), 3, 4, 5, 6, 7, 8, 9 & 10 in *C. striata* and in *C. gachua* an additional fraction 'e' occurred in the region of band 2. In all the three species the region of band 2 showed extensive variation in the number and relative positions of the electrophoretic bands. The bands were distinct and prominent. Depending on the number and relative positions of these bands, 8, 5 and 7 different patterns could be recognised in *C. punctata*, *C. striata* and *C. gachua* respectively. Band 7 was identified as the transferrin in all the three species. Though identical in their electrophoretic mobility, they differed in their physico-chemical properties such as heat sensitivities and salt solubilities. This fraction was a glycolipoprotein in *C. punctata* and a glycoprotein

in the other two species. The oxidase zone was localized in region of band 10, a glycolipoprotein complex in *C. punctata* and *C. gachua*. In *C. striata* band 7 showed both oxidase and peroxidase activity and it was a glycoprotein. In *C. punctata* and *C. gachua* the peroxidase activity was noted in region of band 8.

Although some similarities were demonstrated in the serum-protein pattern of the

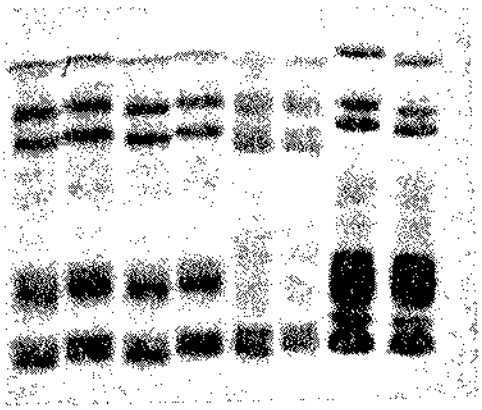

Fig. 3. Electrophoretic protein pattern of serum of *C. gachua*.

three species, enough differences exist in these patterns to distinguish easily one species from the other. The patterns do reflect the close relationship between these species Mc Kenzie and Pairn (1969) found 10 components in the plasma of juvanile Atlantic salmon and Thurston (1967) reported 10–18 fractions in rainbow trout and concluded that the differences were due to a number of factors including hatchery, sex, strain, stress etc. Other workers have found 12 to 16 components in brown trout (Ingram and Alexander, 1977). 13 bands in rainbow trout (Perrier *et al.*, 1973), 8 fractions in *Labeo umbratus* and *Labeo capensis* (Hattingh, 1974) and between 5 and 9 major protein fractions in *Leuciscus leuciscus* (Harris, 1974) all of which were resolved on polyacrylamidae gel electrophorresis.

It is not clear whether the variation in the banding pattern in region 2 represented genetic heterogeneity or polymerisation of the protein molecules to different extents. Further genetic and biochemical studies are needed to elucidate the nature of these bands. They could be compared with albumins on the basis that they have low molecular weight, fast mobility and are quantitatively predominant and could bind iron like human albumins. These bands may be called albumin-like fractions (Harris, 1974) or para albumin (Perrier *et al.*, 1974). The weakly stained component 1 could then be the prealbumins. Harris (1974) reported 2 prealbumin fractions in dace sera.

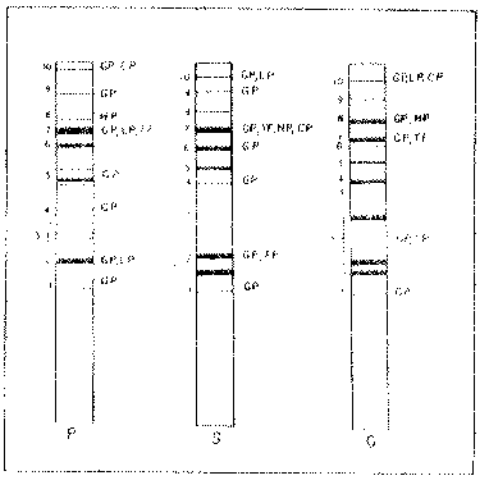

Fig. 4 Diagrammatic representation of the serum proteins of *C. punctata* (P); *C. striata* (S); and *C. gachua* (G); GP, glycoprotein; LP, lipoprotein; TF, Transferrin; CP, Ceruloplasmin; HP, Haptoglobin.

Intraspecies variations in these three species while it exists, is less than interspecies variations. In fact it was our aim to characterise the serum proteins which could be used to study genetic variability among the members of these species. Some fractions in particular the 'albumin-like' fractions of the three species did show variable patterns which could be grouped into few types but in no one species was it possible to ascertain the variability as genetic by observing the phenotypes alone. In the absence of genetic crossing and from the fact that physiological factors (Booke, 1964) can influence the serum

protein patterns, it cannot be said with firmness whether individual variations are due to genetic variation or due to physiological factors.

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