SERUM PROTEIN POLYMORPHISM IN GREY MULLET (*Mugil cephalus* Linn.)

by

Veravat Hongskul
Research Biologist
Marine Fisheries Laboratory, Department of Fisheries
Bangkok, Thailand

ABSTRACT

Starch-gel electrophoretic analysis of plasma and serum protein samples of the Australian grey mullet (*Mugil cephalus* Linnaeus) indicated ten protein components. Polymorphism of Component III with three phenotypes, designated A, B, and AB, were demonstrated. The Hardy-Weinberg analysis of the data suggested that the three phenotypes form a system determined by two alleles, Mc^a and Mc^b respectively.

INTRODUCTION

Serum protein polymorphism has been demonstrated by gel electrophoresis in many species of fish. The transferrin and esterase systems are summarized in Table I. Møller and Naevdal (1966) postulated that serum protein polymorphism was common in fishes and could be used in the study of fish subpopulations. This has been shown by Fujino (1968) and Fujino & Kang (1968 a,b) in a study on skipjack tuna.

This paper presents the results of a preliminary investigation of genetic polymorphism of the serum proteins of the Australian grey mullet (*Mugil cephalus* Linnaeus). The ultimate aim is to compare the Australian results with mullet obtained from Thai waters.

MATERIALS AND METHODS

Mullet Blood Samples

Serum or plasma (obtained by centrifugation of whole blood collected in the Modified Alsever's solution, Sprague & Vrooman, 1962) samples included 37 specimens taken from the Stradbroke Island, Queensland, 73 specimens from the Marineland, Southport, Qld., 7 specimens from McDonald River, New South Wales, and 10 specimens taken from Pineapple Bay, Western Australia. Thus, a total of 127 blood samples were obtained from July to November 1967.

Proc. Indo-Pacific Fish. Coun., 13(II): 107-112.

Blood specimens were taken immediately after capture of the fish by direct puncture of the caudal artery or vein. The blood was allowed to flow into a clean dry tube and after clotting it was centrifuged to separate the serum. The serum was pipetted into a small tube and stored at -20°C until the time of analysis.

Table I

Serum Protein Polymorphism in Fishes

Common name	Scientific name	Serum system *		Reported by
Bigmouth buffalo-fish	*Ictiobus cyprinellus*	Tf	Es	Koehn & Johnson (1967)
Bonito	*Sarda chiliensis*	Tf		Barrett & Tsuyuki (1967)
Brown trout	*Salmo trutta trutta*		Es	Nyman (1965 a, b)
Carp	*Cyprinus carpio*	Tf		Creyssel, *et al.* (1964; 1966); Møller & Naevdal (1966)
Cod	*Gadus morhua*	Tf		Møller & Naevdal (1966)
Pond crucian	*Cryprinus carassius carassius*		Es	Nyman (1966 a, b)
Tuna:				
Albacore	*Thunnus alalunga*	Tf		Barrett & Tsuyuki (1967)
			Es	Fujino & Kang (1968 b)
Bigeye	*Thunnus obesus*		Es	Sprague (1967); Fujino & Kang (1968 b)
Skipjack	*Katsuwonus pelamis*	Tf		Sprague & Fujino (1965); Barrett & Tsuyuki (1967); Fujino & Kang (1968 a)
			Es	Fujino & Kang (1968 b)
Southern bluefin	*Thunnus maccoyii*	Tf		Fujino & Kang (1968 a)
			Es	Sprague (1967)
Yellowfin	*Neothunnus macropterus*	Tf		Sprague & Fujino (1965)
	Thunnus albacares	Tf		Barrett & Tsuyuki (1967); Fujino & Kang (1968 a)
			Es	Sprague (1967); Fujino & Kang (1968 b)

* Tf: Serum transferrins
Es: Serum esterases

Electrophoresis and Staining

The sera and plasma were subjected to the horizontal starch-gel electrophoresis in a discontinuous buffer (Ashton and Braden, 1961), pH 8.5, for four hours at 400 volts. Serum protein bands were identified by staining the gel with 0.1% Nigrosine for one hour, as well as staining with amido black (Smithies, 1955).

RESULTS AND DISCUSSION

The electropherogram obtained (Fig. 1) showed that the serum and plasma protein of the grey mullet consisted of ten components. The proteins were designated Components I-X in order of decreasing mobility to anode (Fig. 1 B). The Components II, III and IV were present in reasonably high concentrations while the remainder were in much lower concentrations. According to Drilhon (1960), it is impossible to identify with certainty the nature of the protein in each fraction without carrying out chemical analyses. Therefore, the nature of each protein component is not known at the present time.

However, two-dimensional electrophoresis (Poulik and Smithies, 1958) indicated the homogeneity of Components II and IV (Fig. 2) but revealed the heterogeneity of Component III to be the same as that demonstrated by one-dimensional electrophoresis (Fig. 2 - on the right).

The variations in the electrophoretic patterns observed in the samples were those of Components II, III and V. The variation of Component II was rare and was found only in two samples from the Marineland. Most sera contained a fast-moving Component II protein. These two sera from Marineland lacked this protein and had instead a slower migrating protein (Fig. 1 A - Sample No. 6). The Component V was present only in a few samples from Queensland and New South Wales. Only the variation of Component III, as shown schematically in Fig. 3, was widely distributed among samples.

Three phenotypes of Component III were found. Sera contained either a single fast-moving band, a single slow-moving band, or a combination of the two bands. The observed frequencies of these types, designated A, AB and B, are shown in Table II. The relative proportions of the three phenotypes are in close agreement with the Hardy-Weinberg expectation assuming the existence of two alleles in the frequencies $Mc^a = 0.645$ and $Mc^b = 0.355$ (See: Barrett and Tsuyuki, 1967; Fujino and Kang, 1968 a).

Table II

Hardy-Weinberg Analysis of Mc System in the Grey Mullet

Frequency	Phenotype			Total	D.F.	Chi square	P
	A	AB	B				
Observed	42	58	10	110	1	2.5077	0.05
Expected	45.8	50.4	13.8	110			

Alleles: $Mc^a = 0.645$
$Mc^b = 0.355$

The variation of Component III as mentioned above is probably transferrin polymorphism but yet to be tested by Fe^{59}.

Polymorphism of Component III was not found in the samples taken from Western Australia. This might indicate the geographically intraspecific variation among the mullets collected from the Pacific coast and the Indian Ocean coast of Australia but the number of samples is too small for any real conclusion to be drawn. On the other hand, the study on the electrophoretic patterns of mullet's serum proteins in Thai and adjacent waters should be initiated in the future, in order to clarify the relationship between the different fish populations.

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude and appreciation to Dr. T.K. Bell, Department of Preventive Medicine, School of Veterinary Science, University of Queensland, for his interest, encouragement and valuable suggestion in this study and also criticism of the manuscript; to Professor J.M. Thomson, Department of Zoology, University of Queensland, for his kind arrangement in the collection of the blood samples; to Mr. C.W. Hooker, N.S.W. State Fisheries Laboratory, and Mr. E.H. Barker, Department of Fisheries and Fauna, Western Australia, for collecting the specimens in N.S.W. and W.A.; to the Manager and staff members of the Marineland, Southport, Queensland, for their cooperation and assistance in collecting the blood samples there; and to Mr. Max Smith for his technical assistance.

Acknowledgements also due to Dr. K. Fujino, Bureau of Commercial Fisheries Biological Laboratory, Honolulu, Hawaii, for his helpful advice; to East-West Centre, University of Hawaii, and to Mr. J.C. Marr, Director of the U.S. Bureau of Commercial Fisheries, Hawaii, for the assistance which enabled the author to visit the Honolulu Laboratory.

This investigation was supported (in part) by the Australian Government's fellowship under the Colombo Plan.

REFERENCES

Ashton, G.C., and A.W.H. Braden (1961). Serum Beta-globulin Polymorphism in Mice. Australian J. Exp. Biol. Med. Sci., 14: 248-253.

Barrett, I., and H. Tsuyuki (1967). Serum Transferrins Polymorphism in Some Scombroid Fishes. Copeia, (3): 551-557.

Creyssel, R., P. Silberzahn, G. Richard and Y. Manuel (1964). Étude du Sérum de Carp (*Cyprinus carpio*) par Électrophorèse en Gel d'Amidon. Bull. Soc. Chi. Biol., 46: 149-159.

Creyssel, R., G.B. Richard and P. Silberzahn (1966). Transferrin Variants in Carp Serum. Nature, 212:1362.

Drilhon, A. (1960). L'Apport de l'Électrophorèse en Gel d'Amidon à l'Étude des Protéines Sériques des Poissons. Bull. Inst. Océanogr. (Monaco). (1168): 1-30.

Fujino, K. (1968). Skipjack Subpopulation Identified by Genetic Characteristics. Proceeding of the Symposium on the Results of CSK, Honolulu, Hawaii, 29 April - 2 May 1968. (In press).

Fujino, K. and T. Kang (1968a). Transferrin Groups of Tunas. Genetics, 59: 79-91.

---------- (1968b). Serum Esterase Groups of Pacific and Atlantic Tunas. Copeia, (1): 56-63.

Koehn, R.K. and D.W. Johnson (1967). Serum Transferrin and Serum Esterase Polymorphisms in an Introduced Population of the Bigmouth Buffalofish, *Ictiomus cyprinellus*. Copeia, (4): 805-808.

Moller, D. and G. Naevdal (1966). Serum Transferrins of Some Gadoid Fishes. Nature, 210(5033): 317-318.

Nyman, L. (1965a). Species Specific Proteins in Freshwater Fishes and their Suitability for a Protein Taxonomy. Hereditas (Sweden), 53: 117-126.

--------- (1965b). Inter- and Intraspecific Variation of Proteins in Fishes. Ann. Acad. Reg. Sci. Upsaliensis, 9: 1-18.

Poulik, M.D. and O. Smithies (1958). Comparison and Combination of the Starch-gel and Filter-paper Electrophoretic Methods Applied to Human Sera: Two-dimensional Electrophoresis. Biochem. J., 68(4): 636-643.

Smithies, O. (1955). Zone Electrophoresis in Starch-gels: Group Variations in the Serum Proteins of Normal Human Adults. Biochem. J., 61: 629-641.

Sprague, L.M. (1967). Multiple Molecular Forms of Serum Esterase in Three Tuna Species from the Pacific Ocean. Hereditas, 57(11): 198-204.

Sprague, L.M. and K. Fujino (1965). Serum Polymorphisms of the Skipjack (*Katsuwonus pelamis*) and the Yellowfin (*Neothunnus macropterus*) Tunas. Genetics, 52(2, Pt. 2): 476-477.

Sprague, L.M. and A.M. Vrooman (1962). A Racial Analysis of the Pacific Sardine (*Sardinops caerulea*) Based on Studies of Erythrocyte Antigens. Ann. N.Y. Acad. Sci., 97: 131-138.

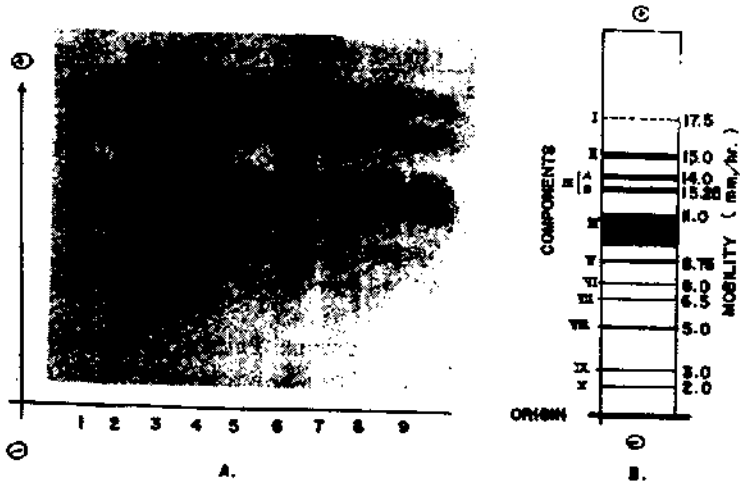

FIG. 1. Electrophoregram of mullet's serum proteins (A) and their schematically mobilities of each component

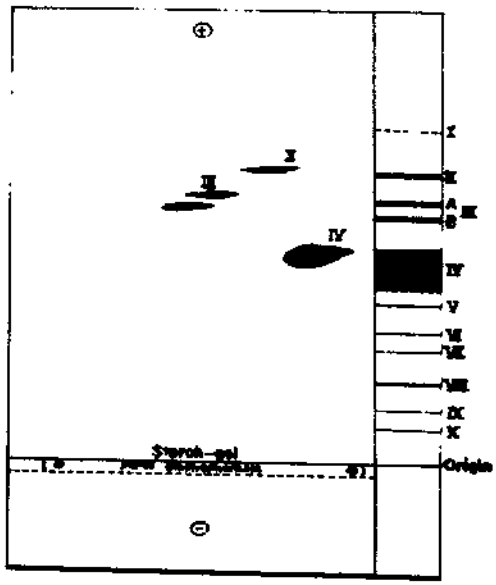

Fig. 2. Two-dimensional electrophoretic pattern of mullet's serum, compared with one-dimensional electrophoresis (right)

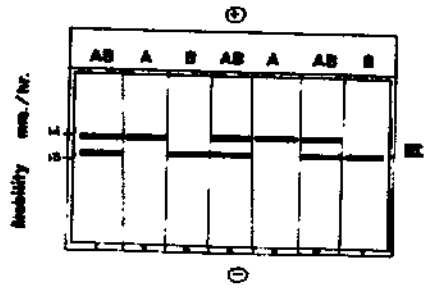

Fig. 3. Three phenotypes of Component III and their mobilities