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Evidence for Genetic Variation in the Sarcoplasmic Protein of *Nemipterus peronii* (Valenciennes)

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Abstract

The sarcoplasmic proteins of *Nemipterus peronii* were electrophoretically analyzed using the isoelectric focusing (IEF) technique. A polymorphic zone at pH 3.69-3.79 was observed, giving three phenotypes: A, AB and B. The expected frequencies of the occurrence of each of the phenotypes, according to the Hardy Weinberg law, agreed with the observed frequencies.

Introduction

The Nemipterids or threadfin breams are small to moderately sized fishes commonly found throughout the Indo-Pacific region (Eggleston 1970). Lately, these species have been receiving increasing attention because of their commercial importance, specifically in the manufacture of surimi and surimi-based products. Among the most abundant species of the genus, *Nemipterus* is the rosy threadfin bream or *Nemipterus peronii*. Sainsbury and Whitelaw (1984) and Eggleston (1972) have conducted thorough studies on its biology. However, little is known about its sarcoplasmic proteins and its characteristics.

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Polymorphisms of the sarcoplasmic proteins in some fish species have been detected electrophoretically (Moav et al. 1976; Kirpichnikov 1981). The isoelectric focusing (IEF) technique, which separates protein as bands or electrophoretic patterns, has been applied to fish species identification (Yamada and Suzuki 1982; Ng et al. 1986) and detection of genetic variations (Taniguchi et al. 1982). This study was designed to analyze polymorphisms found in the IEF patterns of the sarcoplasmic proteins of *N. peronii*. It aims to confirm the observed polymorphism in *N. peronii* as a form of genetic variation.

Materials and Methods

Samples of *N. peronii* (12-24 cm) were purchased from Punggol Fishing Port, Singapore, in May-June 1992, and transported in ice to the Marine Fisheries Research Department (MFRD) laboratory. The samples were washed, photographed, individually wrapped in linear low density polyethylene film, and stored at -20°C. Most samples were used for analysis within two weeks.

Protein extraction was carried out by homogenizing 1 g muscle from the anterio-dorsal part of the fish with 5 ml distilled water in mortar and pestle (1:5 fish:water ratio). The homogenate was centrifuged (Beckman J2-21, USA) at 8,000 rpm for 15 min and the filtrate collected and stored in Nunc vials (InterMed, Denmark) at -70°C until required.

The solutions used for isoelectric focusing were generally adapted from Ng et al. (1986). The anode solution was 1 M H_3PO_4 and the cathode solution was 1 M NaOH. The fixing solution comprised of 17.25 g sulfosalicylic acid dihydrate, 57.5 g trichloroacetic acid and 150 ml methanol and made up to 500 ml with distilled water. The destaining solution was made by dissolving 150 g acetic acid in some water, then adding 500 ml ethanol. The whole mixture was made up to 2,000 ml with distilled water. The staining solution was made by dissolving 0.23 g Coomassie Blue R-250 in 200 ml destaining solution, while the preserving solution was made by adding 50 ml glycerol to 500 ml destaining solution.

The isoelectric focusing was conducted in an electrophoresis unit (LKB Multiphor II Model 2117, Sweden) equipped with a thermostatic circulator (LKB Multitemp II Model 2219, Sweden) with

the following setting: power at 25 watts; current at 50 amps; voltage at 1,500 volts; and the cooling platen at 5°C.

A few drops of kerosene were pipetted onto the cooling platen and the screen print template was then placed on the platen. Commercially prepared polyacrylamide gel (AMPHOLINE PAGplate, pH 3.5-9.5, size 245 x 110 x 1 mm) was carefully positioned on top of the template, making sure that no air bubbles were trapped beneath the gel. The electrode strips were soaked evenly with 1M H_3PO_4 and 1M NaOH and placed in the anodic and cathodic ends of the gel, respectively.

The thawed sample extracts were carefully applied onto the gel using strips of filter paper (5 x 10 mm). Commercial pI marker (Pharmacia, USA) containing 12 protein standards, was also applied onto the gel using the applicator strip. After sample application, the unit was run for 30 min, after which the strips were taken off the gel and the isoelectric focusing was continued for another 75 min.

Immediately after electrofocusing, the gel was immersed in fixing solution for 60 min. Using destaining solution, the gel was rinsed twice and then finally immersed in destaining solution for 60 min. The gel was stained using preheated (50°C) Coomassie Blue Solution for 5 min and was then rinsed twice with destaining solution. Further destaining was done until a clear background was achieved. After destaining, the gel was immersed in preserving solution for 60 min, dried in a 45°C incubator (MEMMERT, West Germany) for 45 min and mounted in a mylar sheet for protection.

Densitometric reading (LKB UltroScan XL Laser Densitometer, Sweden) was conducted to determine the apparent pI values of the samples by comparing the distance from the cathodic end to the peak of each protein band in the samples with those in the protein standards. The densitometer was set at a peak height limit of 0.18 au and at peak width limit of 1.00 mm for all the samples examined.

Results and Discussion

Samples of *N. peronii* were analyzed using the isoelectric focusing (IEF) technique which revealed the existence of three unique electrophoretic patterns in the samples of *N. peronii* (Fig. 1).

Visually, the protein bands, including the minor ones, in almost all of the samples analyzed were found to be similar, with the most

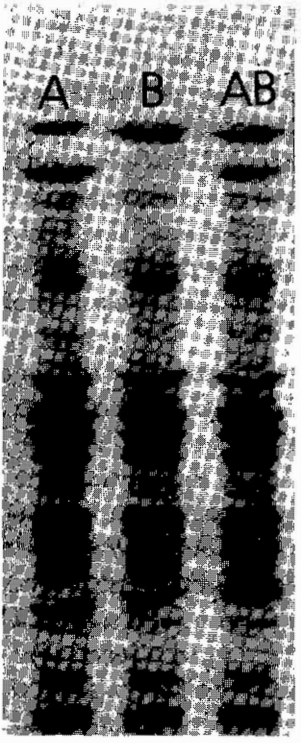

Fig. 1. Isoelectric focusing (IEF) patterns of the three phenotypes (A, B, AB) in *Nemipterus peronii*.

prominent acidic bands (pH<5.00) detected at pH 3.67, 3.69, 3.79, 4.13 and 4.75. However, protein polymorphism was observed between pH 3.69 and 3.79, wherein two different major bands, A and B, were present. The densitometric analyses of these protein bands further illustrated these observed differences (Fig. 2).

In this study, the pH range 3.69-3.79 was considered as a polymorphic zone, with all the samples of *N. peronii* analyzed classified into three phenotypes (A, AB and B) based on the two bands detected in this pH range. As schematically presented in Fig. 3, phenotype A has one band at pH 3.79, phenotype B has a band at pH 3.69 and phenotype AB has both these bands. As such, it can then be supposed that the samples analyzed have two alleles A and B, and three underlying genotypes AA, AB and BB, with an expected segregation ratio of 1:2:1.

To determine whether this proposed ratio was likely to be real or merely due to chance errors in sampling, the Hardy Weinberg principle was used, under the basic assumptions of the existence of random mating among the samples in a population, that the supposed genotypes are equally viable, and that the genes in this species have no differential mutability (Richarson et al. 1986).

Using the computed allelic frequency of homozygote A and the observed frequencies of each phenotype out of the sample population examined, the Hardy-Weinberg expected frequencies were calculated and presented in Table 1. The

Table 1. Observed polymorphism of the sarcoplasmic proteins of *Nemipterus peronii* at pH range 3.69-3.79.

Observed phenotypes	Observed frequencies*	Hardy-Weinberg expectations**
A	16	15.8
AB	27	27.4
B	12	11.8

* Total number of samples = 55
** Allelic frequency of phenotype A = 0.536
Chi-square value = 0.011

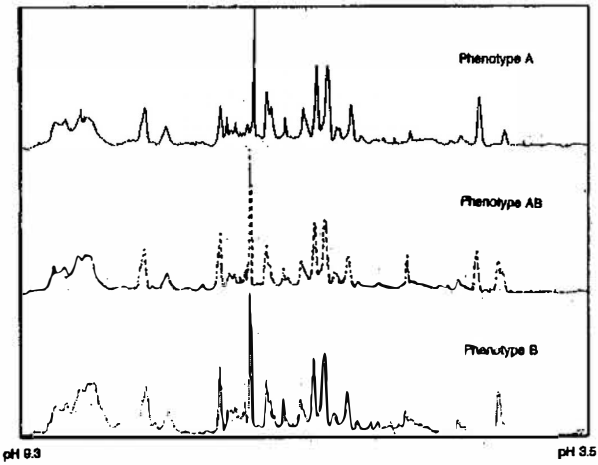

Fig. 2. Densitometric readings of the isoelectric focusing (IEF) patterns of three polymorphic patterns in *Nemipterus peronii*.

expected values for homozygotes A and B and the heterozygote AB, according to the Hardy-Weinberg law agreed with the observed allelic frequencies, as shown by the chi-square test for heterogeneity ($p \geq 0.99$).

The observed genetic variation in *N. peronii* may be attributed to the abundance of this species which is well documented (Eggleston 1968; Sainsbury and Whitelaw 1984). In addition to this, the observed genetic variation in *N. peronii* in this study seemed not at all surprising, considering the enormous number of publications dealing with protein variability within fish species, as reviewed by Kirpichnikov (1981), and the studies on the existence of genetic variation in fish species (Wilkins and Iles 1966; Taniguchi et al. 1982; Shaklee and Salini 1983, 1985).

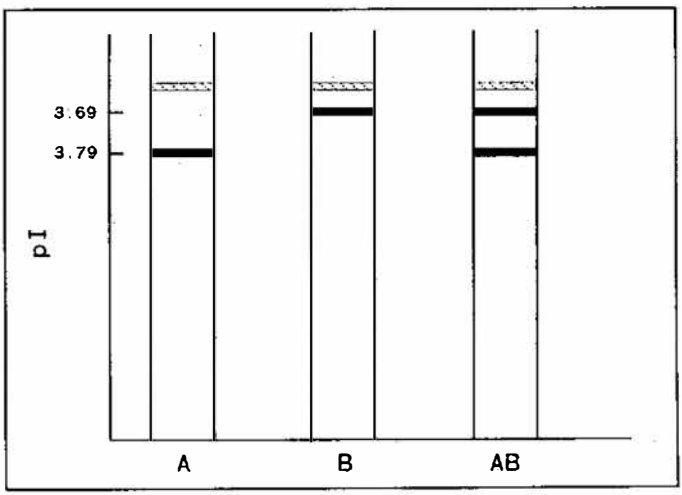

Fig. 3. Schematic representation of the three phenotypes in *Nemipterus peronii*.

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