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The Biochemical Population Genetics of Redfishes (*Sebastes*) Off Southern Newfoundland

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ABSTRACT

Electrophoretic analysis of liver enzyme variants from redfish specimens classified as Sebastes marinus and Sebastes fasciatus on gasbladder musculature and morphological characters provides clear evidence that these taxons represent true biological species. We also present evidence that S. fasciatus and S. mentella are genetically distinct and that S. mentella is more closely related to S. marinus than to S. fasciatus. Preliminary work suggests that it will soon be possible to devise a biochemical genetic protocol that will readily and accurately distinguish specimens of the three sibling redfish species in the northwestern Atlantic.

INTRODUCTION

The taxonomic status of northwestern Atlantic redfishes has been controversial for past decades. Templeman and Sandeman (1957) pointed out two distinct "types" of redfish in this area which may correspond to the European species, Sebastes marinus (L.) and S. mentella Travin. Specimens are classified as follows: the "mentella-type redfish" is bright red in colour with a well-developed bony protrusion on the lower jaw and a large eye, the "marinus-type redfish" is orange to orange-red in colour with a poorly-developed protrusion on the lower jaw and a relatively small eye. Intermediate specimens are quite common. The situation has become further complicated by Barsukov (1968) who recognises two species of "mentella-type redfish" off eastern North America, S. mentella Travin and S. fasciatus Storer. Although morphological differences among redfishes were described by Barsukov (1972), Barsukov and Zakharov (1972), Litvinenko (1974, 1980), Templeman (1980) and Ni (1981a, b), opinions are still expressed that the morphological differences are merely geographic variations within the same species rather than among species (NAFO, 1981).

The rapid increase in the commercial utilization of Northwestern Atlantic redfishes in past decades and the consequent need to develop an effective management policy for the resource has focussed attention on redfish biology. It is of course axiomatic that little progress in biological understanding can be made until the taxonomic problems have been resolved. This report is an attempt to contribute to the solution by providing an analysis of biochemical genetic variation among redfish samples taken off southern Newfoundland.

MATERIALS AND METHODS

Specimens were collected by otter trawling during a research cruise of the 'A. T. Cameron' in June, 1981, to southern Newfoundland waters (3Ps). Specimens were collected at depths from 147 to 472 m. Temperatures were from 2.1 to 5.8°C. Classification of specimens into species was based on extrinsic gasbladder musculature as described by Ni (1981a) and Power and Ni (1982). Heart, liver and skeletal muscle samples were dissected from each specimen and individually frozen. The carcasses were then preserved in 10% formalin for double checking the gasbladder musculature if necessary. The fork lengths ranged from 23 to 58 cm for S. marinus, from 13-42 cm for S. fasciatus and from 30-41 cm for S. mentella .

In the laboratory, tissue specimens were homogenized with an equal volume of 30% dimethyl sulphoxide, 70 mM 'tris', pH 7.5, and homogenates were centrifuged at 4000 X G for 15 min.

The buffer system for starch gel electrophoresis was 135 mM 'tris', 45 mM citric acid, pH 7.0, which was used full strength in the electrode vessels and diluted 1 to 15 for gel preparation (Ayala et al. 1972). Starch gels (15%) were prepared in 180 mm X 180 mm X 6 mm plastic moulds. Samples were applied to the gels on 5 mm squares of 'Whatman No. 3' filter paper, and horizontal electrophoresis was conducted at 150 v and room temperature with forced air cooling for 5 hr. This system was used for LDH, MDH, IDH, PGI, 6-PGD and PGM resolution. All these enzymes from redfishes were found to migrate anodally with this system.

The buffered staining mixture for dehydrogenases, PGI and PGM was 100 mM 'tris', 10 mM $MgCl_2$, containing 100 mM DL-lactate, lithium salt (for LDH staining), or 50 mM L-malic acid (for MDH staining), or 10 mM 6-phosphogluconic acid, trisodium salt (for 6-PGDH staining), or 15 mM DL-isocitric acid, trisodium salt and 100 mM manganous sulphate (for IDH staining), or 10 mM D-fructose-6-phosphate (for PGI staining), or 10 mM glucose-1-phosphate, disodium salt (for PGM staining), and adjusted to pH 8.0 with HCl or NaOH as required. Gel slices were stained for LDH, or MDH by incubation in the dark in 100 ml of the appropriate buffered staining mixture containing 30 mg NAD, 30 mg MTT and 5 mg PMS. Gel slices were stained for 6-PGDH, IDH, PGI and PGM with a Whatman 1 chromatography paper overlay containing 5 mg NADP, 2 mg MTT, 0.5 mg PMS and (for PGI and PGM only) 10 units glucose-6-phosphate dehydrogenase (Sigma Type XII) in 5 ml of the appropriate substrate. SOD appeared as light spots against a darker blue background when any of these gels were exposed to strong light after specific staining.

An alternative buffer system (Ashton and Bradon, 1961) was used for typing esterase variants by polyacrylamide gel electroporesis. Polyacrylamide gels (6.65% acrylamide, 0.35% N,N'-methylene-bis-acrylamide) were prepared in 180 mm X 180 mm X 6 mm plastic moulds with a buffer containing 1.4 g/l citric acid, 5.58 g/l 'tris', 1.18 g/l boric acid and 0.12 g/l lithium hydroxide. The vessel buffer contained 11.8 g/l boric acid and 1.2 g/l lithium hydroxide. Electrophoresis was conducted at 250 v until the 'front' had migrated 100 mm past the sample origin. Gels were sliced horizontally and one section was stained for esterase with 1-naphthyl acetate and Fast Red TR salt: 5 mg 1-naphthyl acetate was dissolved in 5 ml warm ethanol, diluted to 100 ml with water, and mixed with another 100 ml of water containing 50 mg Fast Red TR salt. The other section was stained with the fluorogenic substrate 4-methylumbelliferyl acetate: 10 mg 4-MU acetate was dissolved in 2 ml boiling ethanol, diluted to 100 ml with 100 mM acetate buffer pH 5.2, and poured over the gel. Bands were visualized under long wave UV illumination.

IDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; NAD, nicotinamide adenine dinucleotde; NADP, nicotinamide adenine dinucleotide phosphate;MDH, malate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase; PGI, phosphoglucose isomerase; PGM, phosphoglucomutase; PMS, phenazine methosulphate; 'tris', 2-amino-(hydroxymethyl)propane-1,3-diol; SOD, superoxide dismutase.

RESULTS AND DISCUSSION

Some of the enzymes investigated were monomorphic for all specimens and in consequence are unimportant as systematic characters; these enzymes, LDH, 6-PGDH and PGM, are not considered further in this report. The observed phenotype frequencies for polymorphic enzymes are listed in Table 1.

Malate dehydrogenase (MDH) occurs in two forms, cytoplasmic (supernatant) and mitochondrial. The mitochrondrial MDH was monomorphic for all redfish specimens examined. The possession of two loci for cytoplasmic MDH is a characteristic of fishes and amphibians. The relative staining of the cytoplasmic MDH isozymes varies with tissue type; the products of Mdh-A and Mdh-B loci are strongest in liver and skeletal muscle, respectively. The Mdh-A locus of North Atlantic redfish was found to be polymorphic with two alleles, $Mdh\text{-}A^1$ and $Mdh\text{-}A^2$, determining three phenotypes, $MHD\text{-}A^1$, $MDH\text{-}A^1/MDH\text{-}A^2$, and $MDH\text{-}A^2$. There is a marked difference in the frequencies of the two Mdh-A alleles between S. marinus and S. fasciatus; the frequency of $Mdh\text{-}A^1$ being 0.79 ± 0.05 and 0.15 ± 0.04, respectively.

Electrophoresis of liver homogenates revealed two classes of polymorphic esterases: EsA which hydrolyses 1-naphthyl acetate, and EsB which hydrolyses 4-methylumbelliferyl acetate. The EsA polymorphism also demonstrates biochemical genetic differences between S. marinus and S. fasciatus: the frequency of EsA^1 is 0.93 ± 0.03 in S. marinus and 0.58 ± 0.06 in S. fasciatus. The frequency of EsB^1 is very similar for both S. marinus and S. fasciatus, 0.45 ± 0.07 and 0.50 ± 0.06, respectively.

SOD-typing also demonstrates that S. marinus and S. fasciatus in southern Newfoundland waters behave as good biological species. While most specimens of either species are phenotypicaly SOD^2 there is a tri-allelic polymorphism involving SOD^1, SOD^2 and SOD^3. Variant phenotypes (N = 16) were only found among specimens previously identified as S. fasciatus on the basis of gasbladder musculature.

The IDH polymorphism is of little utility as a biochemical discriminator as the three phenotypes occur at the same frequency in both species.

Only 5 specimens of S. mentella were obtained from this collection, so it would be improper to draw any conclusions on the merits of the various polymorphisms to discriminate between S. mentella and the two other redfish species in this area. However, it would appear from data available to us from specimens taken elsewhere that S. mentella may be much more closely related to S. marinus than to S. fasciatus. For example, $Mdh\text{-}A^1$ and EsA^1 are the commonest alleles in S. marinus and S. mentella, while $Mdh\text{-}A^2$ and EsA^2 are commonest in S. fasciatus. If this observation is verified, then the category of "mentella-type redfish" used in current stock assessment is biologically meaningless and likely to produce management decisions that are invalid.

The best polymorphisms found so far for discriminating between S. fasciatus and S. marinus appear to be MDH and EsA. Eighty-two percent of S. marinus specimens were $EsA^1/MDH\text{-}A^1$ or $EsA^1/MDH\text{-}A^1,A^2$ while only 13% of S. fasciatus specimens belonged to these phenotypic categories. On the other hand, 78% of S. fasciatus were $MDH\text{-}A^2$ but only 4% of S. marinus specimens. We are confident that it will soon be possible to devise a biochemical genetic protocol that will discriminate readily between individuals of the sibling species of Sebastes in our area, and so permit a more rational utilization of the resource.

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Table 1. Observed phenotype frequencies for common enzyme polymorphisms in redfishes (Sebastes) collected off southern Newfoundland (area 3Ps). The frequencies in parentheses are those expected for genetic equilibrium.

Locus	Phenotype	Sebastes marinus	Sebastes fasciatus	Sebastes mentella
Mdh-A	1	17[17.3]	3[0.8]	5
	1-2	10[9.4]	5[9.4]	0
	2	1[1.3]	29[26.8]	0
EsA	1	24[24.2]	11[12.5]	3 [2.25]
	1-2	4[3.7]	21[18.0]	0 [1.5]
	2	0[0.1]	5[6.5]	1 [0.25]
Idh	1	30[30.5]	35[34.7]	5
	1-2	9[7.96]	9[9.66]	0
	2	0[0.52]	1[0.67]	0
SOD	1-2	0	1[0.6]	0
	2	35	25[23.7]	4
	2-3	0	11[14.3]	0
	3	0	4[2.2]	0
EsB	1	9[5.83]	9[9.03]	2 [2.25]
	1-2	8[14.34]	18[18.0]	2 [1.50]
	2	12[8.83]	9[9.0]	0 [0.25]