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Biochemical Population Genetics of Redfishes (*Sebastes*) off Newfoundland

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Abstract

Electrophoretic analysis of liver enzyme variants from redfish specimens, classified as *Sebastes marinus* and *Sebastes fasciatus* on the basis of meristics and gasbladder musculature, provides clear evidence that these taxa are valid biological species. It is also evident that *S. fasciatus* and *S. mentella* are genetically distinct and that *S. mentella* is more closely related to *S. marinus* than to *S. fasciatus*. These preliminary analyses indicate that it may soon be possible to devise a biochemical genetic protocol that will readily and accurately distinguish specimens of the three sibling redfish species in the Northwest Atlantic.

Introduction

The taxonomic status of redfishes in the Northwest Atlantic has been controversial for many years. Templeman and Sandeman (1957) pointed out two distinct "types" of redfish in the region which may correspond to the European species, *Sebastes mentella* Travin and *Sebastes marinus* (Linnaeus). Specimens classified as the "mentella-type" or "beaked" redfish are bright red in color with a well-developed bony protrusion on the lower jaw and a large eye, whereas those classified as "marinus-type" or "golden" redfish are orange to orange-red in color with a poorly-developed protrusion on the lower jaw and a relatively small eye. Specimens with intermediate characteristics are quite common. The situation has become further complicated by the work of Barsukov (1968) who recognized two species of beaked redfishes off eastern North America: *S. mentella* Travin and *S. fasciatus* Storer. Although morphological differences among the redfishes were described by Barsukov (1972), Barsukov and Zakharov (1972), Litvinenko (1974, 1980), Templeman (1980) and Ni (1981a, 1981b), 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 Northwest Atlantic redfishes in recent decades and the consequent need to develop an effective conservation policy for the resource has caused attention to be focused on the biology of redfishes. It is, of course, axiomatic that little progress in biological understanding can be made until the taxonomic problems have been resolved. This paper attempts to contribute to the solution by providing the results of an analysis of biochemical variation among redfishes in samples taken off Newfoundland.

Materials and Methods

The redfish samples, consisting of 63 *S. fasciatus*, 39 *S. marinus* and 5 *S. mentella*, were collected during an otter-trawl survey off southern Newfoundland (St. Pierre Bank) by the research vessel *A. T. Cameron* in June 1981. The specimens were caught in depths from 147 to 472 m, where bottom temperatures ranged from 2.1° to 5.8° C. In addition, 31 specimens of *S. mentella* were obtained from a catch of the commercial trawler *Cape Fox* fishing off southern Labrador (Hamilton Bank) at 307-314 m on 20 September 1982. Classification of the specimens into species, based on extrinsic gasbladder musculature as described by Ni (1981a) and Power and Ni (1982), was carried out independently prior to the biochemical genetic analysis. Heart, liver and skeletal muscle samples were dissected from each specimen and individually frozen. The carcasses were then preserved in 10% formalin for future checking of the gasbladder musculature, if necessary. Prior to dissection and preservation, the specimens were measured as fork length to the nearest centimeter. The length ranges of the specimens were 23-58 cm for *S. marinus*, 13-42 cm for *S. fasciatus* and 26-41 cm for *S. mentella*.

The following abbreviations for various biochemical terms terms have been used throughout the text:

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

In the laboratory, liver specimens were homogenized with an equal volume of 30% dimethyl sulphoxide, 70 mM 'tris', pH 7.5, and the homogenates were centrifuged at 4,000 × 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 × 180 mm × 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-PGDH and PGM resolution. All of these enzymes from redfishes were found to migrate anodally with the system.

The buffered staining mixture for the six enzymes was 100 mM 'tris' and 10 mM $MgCl_2$, with 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 and 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 No. 1" chromatography paper overlay containing 5 mg NADP, 2 mg MTT, 0.5 mg PMS and (for PGI and PGM only) 10 units of 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 electrophoresis. Polyacrylamide gels (6.65% acryalamide; 0.35% N,N'-methylene-bis-acrylamide) were prepared in 180 mm × 180 mm × 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 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 (10 mg 4-methylumbelliferyl acetate 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 ultraviolet illumination.

Results and Discussion

The enzymes, LDH, 6-PGDH and PGM, were found to be monomorphic for all specimens investigated and are consequently unimportant as systematic characters. The observed phenotype frequencies for polymorphic enzymes are listed in Table 1.

Cytoplasmic malate dehydrogenase (MDH; L-malate:NAD oxidoreductase; EC 1.1.1.37) is determined in fishes and amphibians by two genes, Mdh-A

TABLE 1. Observed phenotype frequencies for common enzyme polymorphisms in redfishes (*Sebastes* sp.) off Newfoundland. (Frequencies in parentheses are those expected for genetic equilibrium.)

Locus	Phenotype	*S. marinus*		*S. fasciatus*		*S. mentella*	
Mdh-A	1	25	(25.1)	3	(0.8)	36	(36.0)
	1-2	11	(10.7)	6	(10.4)	0	
	2	1	(1.1)	35	(32.8)	0	
EsA	1	26	(26.1)	9	(10.8)	34	(33.0)
	1-2	4	(3.7)	22	(18.4)	0	(1.9)
	2	0	(0.1)	6	(7.8)	1	(0.03)
Idh	1	30	(30.5)	52	(51.6)	5	
	1-2	9	(8.0)	10	(10.9)	0	
	2	0	(0.5)	1	(0.6)	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	(5.8)	9	(9.0)	2	(2.3)
	1-2	8	(14.4)	18	(18.0)	2	(1.5)
	2	12	(8.8)	9	(9.0)	0	(0.3)

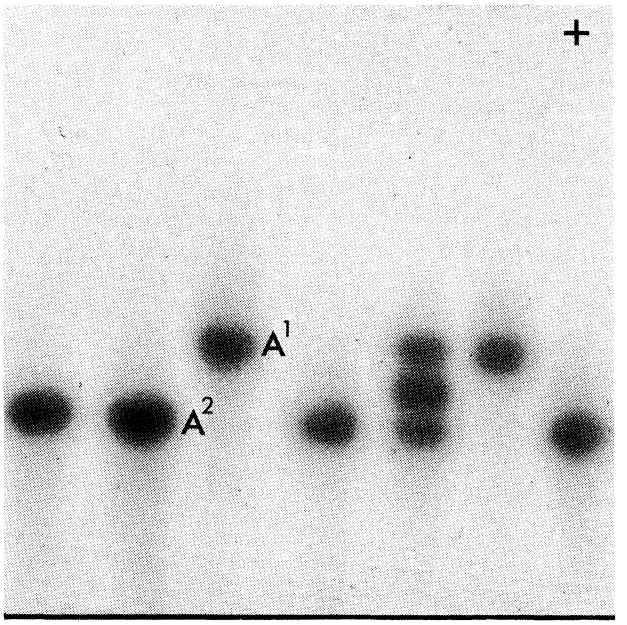

Fig. 1. Starch gel electrophorogram of liver extracts from redfish specimens stained to demonstrate malate dehydrogenase. Three phenotype patterns were observed: a single, anodally-migrating band which is interpreted as the product of a homozygous Mdh-A^1 genotype; a single, less-anodal band, genotype Mdh-A^2; and a 3-banded pattern (MDH-A^1 homodimer, MDH-A^1/MDH-A^2 heterodimer, MDH-A^2 homodimer) in specimens that are Mdh-A^1/Mdh-A^2 heterozygotes.

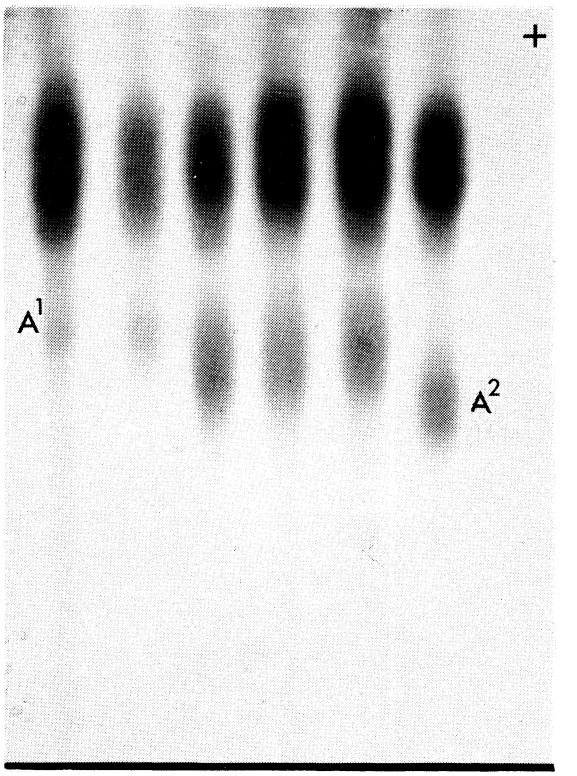

Fig. 2. Polyacrylamide gel electrophorogram of liver extracts from redfish stained to reveal general anodal esterase activity. There is a strongly-staining invariant region and a less-intense polymorphic region (EsA) in which one may observe either a single, fast-migrating band (EsA^1), or a single, slower-migrating band (EsA^2), or a broad, multicomponent band which overlaps EsA^1 and EsA^2 (genotype EsA^1/EsA^2).

and Mdh-B (Bailey *et al.*, 1970). The degree of transcription varies with the tissue type, MDH-A being the dominant isozyme in liver and MDH-B in skeletal muscle. The Mdh-A locus of North Atlantic redfishes was found to be polymorphic with two alleles, designated Mdh-A^1 and Mdh-A^2, determining the three liver MDH-A phenotypes: MDH-A^1, MDH-A^1/MDH-A^2, and MDH-A^2 (Fig. 1). There is a marked difference in the frequencies of the two Mdh-A alleles between *S. marinus* and *S. fasciatus*, the estimated gene frequencies ($\pm$ standard deviation) of Mdh-A^1 being 0.82 ± 0.04 and 0.14 ± 0.04 respectively.

Electrophoresis of liver homogenates revealed two classes of polymorphic esterases: EsA which hydrolyzes 1-naphthyl acetate, and EsB which hydrolyzes 4-methylumbelliferyl acetate. The EsA polymorphism (Fig. 2) also demonstrated biochemical genetic differences between S. *marinus* and *S. fasciatus*, the frequency of EsA^1 being 0.93 ± 0.03 in *S. marinus* and 0.54 ± 0.06 in *S. fasciatus*. The frequency of EsB^1 was very similar for both species, being 0.45 ± 0.07 in *S. marinus* and 0.50 ± 0.06 in *S. fasciatus*.

SOD-typing also demonstrated that *S. marinus* and *S. fasciatus* in southern Newfoundland waters behave as biological species. Although most specimens of either species exhibited the SOD^2 phenotype, tri-allelic polymorphism involving SOD^1, SOD^2 and SOD^3 was evident. 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, because the three phenotypes occur at about the same frequency in *S. marinus* and *S. fasciatus*.

The specimens of *S. mentella*, obtained from the commercial catch off Labrador were not in sufficiently good condition to permit analysis of IDH, SOD or EsB. However, it was quite clear from MDH-A and EsA typing that *S. mentella* and *S. fasciatus* are valid species. The frequency of Mdh-A^2 was 0.86 ± 0.06 in beaked redfish specimens classified as *S. fasciatus* on the basis of gasbladder musculature, but this allele was absent in specimens classified morphologically as *S. mentella*. EsA^2 was common in *S. fasciatus* ($q = 0.46 \pm 0.06$) but rare in S. marinus ($q = 0.07 \pm 0.03$) and absent in *S. mentella*. The observations indicate that *S. mentella* may be more closely related to *S. marinus* than to *S. fasciatus*, because Mdh-A^1 was the most common

allele found in *S. marinus* and *S. mentella*, whereas Mdh-A^2 was more common in *S. fasciatus*. Similarly, EsA^2 was common in *S. fasciatus* but rare in *S. marinus* and probably absent in *S. mentella*. Despite this similarity between *S. mentella* and *S. marinus*, the Mdh-A^1 allele frequencies (1.00 and 0.82 respectively) are sufficiently different ($P<0.001$) to confirm that they are genetically distinct populations.

The significance of these findings to redfish fisheries biology is the biochemical genetic confirmation that the category of "beaked" redfish used in current stock assessments is biologically heterogeneous, and its use is likely to result in management decisions that are invalid. As demonstrated by the preliminary observations reported above, it should soon be possible to devise a biochemical genetic protocol that will discriminate readily between individuals of the sibling species of *Sebastes* in the Northwest Atlantic and so permit a more rational utilization of the resource.

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