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**ELECTROPHORETIC STUDIES OF
REDFISH (GENUS *SEBASTES*) FROM
ICELANDIC AND GREENLAND WATERS**

By

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ABSTRACT

Sebastes spp. from Icelandic and Greenland waters were analysed for biochemical genetic variation by agar gel and starch gel electrophoresis. The main emphasize was put on *S. marinus*, but the oceanic type *S. mentella* from the Irminger sea and *S. viviparus* from coastal Icelandic waters were studied for comparisons. The results were compared to earlier studies on material from East Greenland, Faroe Islands, British sector and off the Norwegian coast (Nedreaas and Nævdal 1991). The *S. marinus* samples were collected southwest of Iceland, on the southeast coast, north of Iceland and from both sides of Cape Farewell. *S. viviparus* were collected from five localities south of Iceland. Haemoglobin and three enzymes in liver and muscle tissue were analysed by use of agar gel and starch gel electrophoresis. Isocitrate dehydrogenase (IDH) were analysed because it shows polymorphism in liver tissue; malate dehydrogenase (MDH), malic enzyme (ME) and haemoglobins first of all because they are species specific. The oceanic type *S. mentella* showed no polymorphism in either of the enzymes and showed an overall picture identical to what has been found in this species in other areas. Idh-genotypes in the samples from Iceland *S. marinus* showed a similar distribution as found around Faroe Islands and the coast of Norway while the Greenland samples were deviating. Intersample differences were indicated also in *S. viviparus*. Pattern of MDH was diagnostic for *S. viviparus*. In all three species were found somewhat unclear variations that will be further studied. The haemoglobin patterns were monomorphic in all three species, and as found before, clearly diagnostic for *S. mentella*, while considerable variation were noted in the material of *S. marinus* from Greenland.

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INTRODUCTION

Inter- and intraspecific biochemical genetic variation among *Sebastes* species were described by Nedreaas and Nævdal (1989) based on material from the Norwegian coast and the Barents Sea. By combination of results of electrophoresis of different protein systems the three species present in the Northeast Atlantic area, *Sebastes marinus*, *S. viviparus* and *S. mentella*, could be clearly distinguished. *S. mentella* differed from the other two species in haemoglobin patterns, while MDH patterns were diagnostic for *S. viviparus*. Liver IDH showed up to be polymorphic for both *S. marinus* and *S. viviparus*, both sharing one common gene, called *Idh-1(100)*. *S. mentella* was found to be monomorphic for this gene. *S. marinus* possessed in addition one frequent and one rare gene, called *Idh-1(60)* and *Idh-1(120)* respectively, while *S. viviparus* similarly possessed a frequent gene, *Idh-1(50)*. Later material from the Faroe Islands (all species), Irminger Sea (*S. mentella*) and East Greenland (*S. mentella* and *S. marinus*) was similarly investigated. While the results of *S. mentella* and *S. viviparus* did not deviate from corresponding results from Norwegian waters, *S. marinus* presented new haemoglobin patterns at East Greenland, and this species also was found to be monomorphic for the *Idh-1(100)* gene in these waters, indicating a clear differentiation between Greenland populations and populations from more eastern areas.

In the present report electrophoretic studies of the *Sebastes* species from Icelandic and Greenland waters are dealt with. The main aim of the study has been to investigate the population structure of *S. marinus* in these waters, or more specifically to see whether the Icelandic populations of this species are related to the populations at the Faroe Islands or at Greenland. A limited material from the other two species have been analysed for comparison, and for studies on population structure of *S. viviparus* in Icelandic waters.

MATERIAL AND METHODS

Six samples of *S. marinus* and five samples of *S. viviparus* were collected during an Icelandic research survey in March 1991. Sampling localities are shown in Figure 1. *S. marinus* was sampled around Iceland while *S. viviparus* was sampled in a restricted area on the southwest coast. One sample of *S. mentella* was collected from the Irminger Sea by the Norwegian fishing vessel 'Langvin'.

Likewise the samples from Greenland were collected during a research cruise in December 1991 conducted by the Greenland Fisheries and Oceans Research Institute. An overview of the samples are given in Table 1.

Sex, age and total length were recorded as a standard procedure. Blood, liver and muscle samples were collected from the fish on board the research vessel, stored on microtest plates and sent in a frozen state to the laboratory in Bergen, where it was kept in an ultrafreezer until analysed. The fish sampled in Greenland waters were deep frozen in the field and sent in a frozen state to the laboratory in Bergen where the tissue sampling was conducted.

Haemoglobins were subjected to agar gel electrophoresis at pH of 8.0. Tissue enzymes

were analysed by starch gel electrophoresis in citric acid/morpholine buffer (AM, pH 6,1). Both methods are described by Nedreaas and Nævdal (1989).

For routine analyses the gels were stained for the following enzymes:

Malate dehydrogenase (MDH), malic enzymes (ME), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH) and phosphoglucose isomerase (PGI). Part of the material was also stained for esterase (EST). Chose of enzymes are based on earlier findings (Nedreaas and Nævdal 1989, 1991).

Accordance between observed and expected Hardy-Weinberg distributions as well as intersample homogeneity were tested using standard X^2 -tests.

RESULTS

HAEMOGLOBINS

The haemoglobins in the samples from Iceland showed the same pattern as described by Nedreaas and Nævdal (1989) from the Barents Sea and the Norwegian coast. The patterns obtained by analyses of haemoglobins of *S. marinus* and of *S. viviparus* are identical, while *S. mentella* showed very different and diagnostic pattern compared to the other two species. The deviating patterns of *S. marinus* previously observed and described in samples from Greenland (Nedreaas & Nævdal 1991) were not seen in the samples from Iceland. However, they were frequent among the material from Greenland. It was not possible to group all specimens into the five different patterns described by Nedreaas and Nævdal (1991), but distribution of "single" and "double" phenotypes in each sample are shown in Table 2. X^2 -homogeneity test after grouping the samples into "east" and "west" groups, gave the following result:

$$X^2 = 0.66$$

$$\text{d.f.} = 1$$

$$P > 0.05$$

showing that no significant differences between East and West Greenland *S. marinus* could be found in this trait.

TISSUE ENZYMES

Isocitrate dehydrogenase, IDH

The overall results of the IDH analyses were in accordance with the inter- and intraspecific as well as intertissue variation of IDH in *Sebastes* species described by Nedreaas and Nævdal (1989). At least two loci are involved. One, called *Idh-2*, is monomorphic (except for a few heterozygotes observed) and well expressed in muscle tissue. The other one, called *Idh-1*, is expressed in liver and is polymorphic in *S. marinus* and *S. viviparus*. In *S. mentella* this locus is monomorphic, except for a few heterozygotes observed.

The distributions of the *Idh-1* genotypes in samples of *S. marinus* are shown in Table 3. In accordance with the previous results (Nedreaas and Nævdal 1989, 1991) three presumed alleles controlling liver *Idh-1* components were found. The slowest moving component is supposed to be controlled by an allele called *Idh-1(60)*, and likewise *Idh-1(120)* is controlling the fastest moving component. Only three *Idh-1(120)*

copies, as heterozygotes, were observed in the material from Iceland, and only in sample number two. The frequencies of *Idh-1(60)* in *S. marinus* around Iceland vary from 0.073 to 0.174. For the whole material the frequency is 0.127, which is on the same level as found among the material from Norwegian water and from the Faroe Islands. However, corresponding frequencies was zero among the material analysed earlier from East Greenland and 0.028 in the material analysed from British Sector northeast of Shetland (Nedreaas and Nævdal 1991). All observed genotype distributions were in accordance with expected Hardy-Weinbergs distributions.

Contrary to earlier findings, the *Idh-1(60)* allele was found in most of the samples from Greenland, see Table 3. In addition the allele *Idh-1(120)* was frequently found. X^2 -test on distributions of alleles showed that the variations among Icelandic samples was not significant ($X^2 = 8.39$, d.f. = 5, $P > 0.05$). Neither it was possible to find differences between areas around Iceland by grouping the samples into three groups; north, southwest and southeast samples ($X^2 = 0.66$, d.f. = 2, $P > 0.05$). However, such variations were found between the East and West Greenland samples ($X^2 = 7.07$, d.f. = 2, $P \sim 0.025$). Pooled samples from Iceland and Greenland also were highly statistically different ($X^2 = 23.3$, d.f. = 2, $P < 0.01$). These differences were mainly caused by the high frequency of the *Idh-1(120)* allele occurring in the samples from Greenland, but also due to different frequencies of the *Idh-1(60)* allele.

In *S. viviparus* only two allelic genes in liver IDH were found. The slowest-moving component, controlled by the allele *Idh-1(50)*, moved more slowly than the slower moving component of *S. marinus*. The second component moved as the most common component of *S. marinus*, and the same designation is given to the controlling gene *Idh-1(100)*. In contrast to *S. marinus* the slowest-moving component is the more common one in *S. viviparus*. Distributions of genotypes are shown in Table 4. The gene frequencies are at the same level as found by Nedreaas and Nævdal (1989) in samples from the Northeast Atlantic. Some differences between samples in genotype distributions and gene frequencies were indicated, but the numbers within samples were too low for statistical tests. However, heterozygote deficiency according to Hardy-Weinberg equilibrium were seen in some samples, Table 4, and in the pooled samples this deficiency was found to be statistically significant ($X^2 = 4.8$, d.f. = 1, $P < 0.05$). A possible explanation of this is that different gene pools of *S. viviparus* exist in these waters, and in some samples as well in the pooled samples Wahlung's effects are seen.

No variation in IDH patterns was found in the sample of *S. mentella*.

Other enzymes

None of the other enzymes studied gave any new results compared to earlier studies (Nedreaas and Nævdal 1989) except that MDH showed a possible polymorphic system in muscle tissue, and this will be further studied by isoelectric focusing and other technique. Likewise the patterns of esterase indicated more genetic variation than revealed until now, and also this enzyme will be further studied.

DISCUSSION

The results of the present study show that the structures of *Sebastes* sp. in the North-Atlantic are more complicated than hitherto recognized. Probable heterogeneity among samples and clear indications of Wahlung's effects in *S. viviparus* show that this species in Icelandic waters is not composed of one random mating population. The sub-units, however, do not seem to be connected to specific geographic areas, because the samples in the present study were collected from nearby fishing stations. Different sex ratio in the different samples indicate sex-dependent migration, which possibly could explain the reason why different sub-units are sampled at close locations.

The main emphasize in the present study, however, was laid on *S. marinus*. The present results on haemoglobin are in close accordance with previous result of Nedreaas and Nævdal (1991) who found a series of double phenotypes in Greenland waters, which only occasionally (Nævdal 1978) had been found elsewhere. The genetic basis of these variations has never been revealed, and the quality of the present material did not allow for exact typing of the specimens according to the descriptions of Nedreaas and Nævdal (1991). However, frequencies of phenotypes deviating from the "normal" pattern could easily be calculated (Table 2). Analyses of fresh haemoglobin would be preferable for closer examinations of the deviating haemoglobin types, and thus the genetic basis could possibly be revealed.

Between sample heterogeneity was also evident concerning distribution of IDH genotypes. In Icelandic water no statistically significant variation was found, although some samples showed deviation close to statistical significance. In the previous investigation *S. marinus* was found to be monomorphic for liver IDH in the Greenland sample. In the present study, however, the *Idh-1(60)* allele was found at rather high frequencies in several samples from Greenland, and in addition the *Idh-1(120)* allele was relatively frequent in some samples. The latter allele has only occasionally been seen before (Nedreaas and Nævdal 1991), and a few copies were also found among the present Icelandic samples. Statistically significant variations were found between samples from East and West Greenland and between the total of samples from Iceland and Greenland respectively. The main contributor to these variations was the frequencies of the *Idh-1(120)* allele, although the frequencies of *Idh-1(60)* also varied significantly.

The single sample of *S. mentella* analysed here did not deviate from earlier studies on this species (Nedreaas and Nævdal 1989, 1991), and thus it seems to be no reason for regarding the oceanic *S. mentella* in the Irminger sea as deviating from *S. mentella* recorded elsewhere.

In conclusion the present study has indicated that the population structure of the *Sebastes* species in the North-Atlantic is complicated. The clearest barrier is found between Iceland and Greenland, although the samples collected in various location within the main areas do not seem to represent extensive random mating populations. Sub-structures are indicated although not connected to certain geographic areas. For further studies on such problems, a closer grid of sampling stations are needed for the whole area of the species' distributions. In addition to the trait used here

(haemoglobins and IDH) new characteristics are needed. Analyses of Mt-DNA and nuclear DNA offer possibilities in this direction.

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REFERENCES

- Nedreaas, K. and Nævdal, G. 1989. Studies of Northeast Atlantic species of redfish (*genus Sebastes*) by protein polymorphism. *J.Cons.int. Explor.Mer.*, 46:76-93.
- Nedreaas, K. and Nævdal, G. 1991. Genetic studies on redfish (*Sebastes spp.*) along the continental slopes from Norway to East Greenland. *ICES J.mar.Sci.*, 48:173-186.
- Nævdal, G. 1978. Differentiation between "*marinus*" and "*mentella*" types of redfish by electrophoresis of hemoglobins. *FiskDir.Skr.Ser.HavUnders.*, 16:359-368.