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POPULATION STRUCTURE OF *AMMODYTES MARINUS* IN THE NORTHEAST ATLANTIC

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ABSTRACT

Samples of the lesser sandeel species *Ammodytes marinus* Raitt were collected from Iceland, Denmark, Scotland, the Faeroe Islands and central and northern North Sea, and analysed for frequency distributions of genotypes of polymorphic enzymes. Main emphasize was laid on the enzymes IDHP, PGI and PGM, which were analysed by standard starch gel electrophoresis with two buffer systems. The chose of enzymes is based upon earlier studies in Scotland, Denmark as well as preliminar analyses at the University of Bergen. The genotype distributions from the different areas were compared by standard statistical methods and interpreted with respect to excistence of different gene pools of this species. The sample from Iceland was the most deviating one compared to the rest of the samples showing that *A. marinus* in the northeast Atlantic consist of more than one panmiktic population.

Keywords: sandeels, electrophoresis, genetic variation, population structure

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INTRODUCTION

The sandeel family Ammodytidae forms a species complex. Five species can be regularly found in Nordic and British waters: the Lesser sandeels (*Ammodytes marinus* and *A. tobianus*), the Smooth sandeel (*Gymnammodytes semisquamatus*), Corbin's sandeel (*Hyperlopus immaculatus*) and the Greater sandeel (*H. lanceolatus*). Sandeels are exploited commercially as bait (on a relatively minor scale), for industrial purpose (meal and oil), and also as fish feed which together represent a major North Sea fishery, with Denmark alone landing around 700,000 tonnes per annum (Popp-Madsen 1994). The ecological importance of the North Sea sandeels probably outweighs their economic value. The three commonest species, *A.marinus*, *A.tobianus* and *H.lanceolatus*, which are themselves amongst the most abundant fish in the North Sea (Yang 1982), feature significantly in the diet of a number of economically important fish such as cod and saith. Sandeels also represent the principal prey of many seabird species, for example Kittiwakes, Arctic terns Puffins and Guillemots. Indeed, the drastic decline in the early 1980's of several Shetland Island seabird colonies has since been attributed to a concomitant reduction in the sandeel populations around the islands (Heubeck 1989, Heubeck and Ellis 1986).

Sandeels are thus very valuable from both an ecological and commercial perspective. Basic and applied research into their biology and their intra/interspecific composition is necessary for an understanding of their ecology as well as for management purposes. Unfortunately, such investigations are often hampered by difficulties in identifying certain sandeel species, particularly when distinguishing between *A. marinus* and *A. tobianus*. However, it has been shown clearly that there excist diagnostic allozyme traits for all the five species (Donaghy *et al.* 1995, Simonsen 1986, Fehervari and Nævdal 1995). These authors also have described polymorphic traits within all species. The aim of the study presented here is to utilize the polymorphic enzyme traits described in *A. marinus* to investigate the population structure of this species, i.e. to find out whether or not the populations in coastal and offshore areas of the North Atlantic share in a common gene pool.

MATERIAL AND METHODS

Samples were obtained from five main regions; Danish coastal waters, the North Sea (central and north), Iceland, Faeroe Islands and the Shetland Islands. After capture the specimens were immediately frozen whole in dry ice or a freezer at -20°C and later in the laboratory stored in a -80°C ultra-freezer until analysed. An overview of the samples are summarised in Table 1. White skeletal muscle and if possible liver were dissected out from part-frozen fish and placed into the wells of a microtitre plate containing an equal volume of distilled water. The tissue was disrupted using ultrasound generated by means of a Vir-sonic apparatus© and then centrifuged at 3000 rpm for 10 minutes. The resulting solution was then absorbed onto filter paper strips and loaded onto 12.5% starch gels for electrophoretic analysis. It was possible to conveniently load up to about 90 such strips per gel. The approximate gel depth was 8 mm. After initial screening using different buffers, histidine buffer, pH 7.0, was used for analyses of AAT, IDH and GPD, and TCB-buffer, pH 8.6, was used for PGM and PGI. Details of the electrophoretic technique can be found in Murphy *et al.* (1990).

Table 1. Account of samples analysed by starch gel electrophoresis for studies on the population structure of *A. marinus*.

Sample	Location	Month of collection	Mean length	Numbers
1. North Sea, central	N057, E006	July 1994	14.5	165
2. Iceland	west coast	Aug. 1994	8.7	171
3. Skagerrak	near Jutland	May 1995	16.7	49
4. North Sea, northern	Viking Bank	June 1995	14.2	161
5. Shetland	Fair Isle	June 1991	9.2	207
6. Faeroe Islands		July 1995	11.8	236
7. Denmark	west coast	July 1995	6.0	60
Total				1149

The following ten enzymes were examined; Aspartate aminotransferase (AAT;2.6.1.1), Creatine kinase (CK;2.7.3.2), Esterase (EST;3.1.1.1) (a-naphtyl acetate specific), Glucose-6-phosphate isomerase (GPI;5.3.1.9), Isocitrate dehydrogenase (IDHP;1.1.1.42), Lactate dehydrogenase (LDH;1.1.1.27), Malate dehydrogenase (MDH;1.1.1.37), Phosphoglucomutase (PGM;5.4..2.2), Glycerol-3-phosphate dehydrogenase (GPD;1.1.1.8) and Sorbitol dehydrogenase (SDH,1.1.1.4). The nomenclature and designations of the alleles followed the conventions laid out by Shaklee *et al.* (1989). For each locus the commonest allele was assigned the number 100, while the other alleles were labeled by their migration relative to the most common one. The allele designations thus indicate a quantitative measure of anodic mobility.

The observed phenotypic distributions were tested for congruence with Hardy-Weinberg expectations of genotypes using a Chi-square test. The same test was used for testing heterogeneity among the samples (contingency tests). Statistical analyses and calculations of genetic distances were carried out using the program BIOSYS Version 1.7 (Swofford and Selander 1981).

RESULTS

Of the ten enzymes examined, five consistently gave relatively clear isoenzyme patterns in at least one of the buffer systems. The number of presumptive loci which could be discerned amounted to about 15. Enzymatic activity of varying degrees could usually be observed using either of the electrophoretic conditions, but for routine analyses the TCB- and histidine buffers were chosen because they produced the most informative results. Short descriptions of all ten enzyme patterns are given below.

Aspartate aminotransferase (AAT; 2.6.1.1)

This enzyme revealed two zones of activity, presumeably controlled by two loci. The more anodic zone (loci called *AAT-2**) displayed a polymorphism with three alleles, and of the expected six genotypes four were actually found. The alleles were designated *AAT-2*80, *100* and **120* respectively. However, due to presence of socalled satellite zone and sometimes weak bands, only the samples giving the technically best results could be reliably typed, and due to this uncertainty no frequency distributions are given in the present report. Polymorphism in this enzyme (two alleles) have also been described by Donaghy *et al.* (1995), while Simonsen (1986) found no variation here.

Creatine kinase (CK; 2.7.3.2)

Weak activity was seen with this enzyme using the histidin buffer system. Only limited genetic informtion could thus be gleaned from the results, but there did appear to be a single invariant locus, which also is in accordance with the results of Donaghy *et al.* (1995). Due to the inconsistent results obtained it was not amenable to phenotypic scoring.

Esterase (EST; 3.1.1.1)

Esterase activity was described in *A. marinus* by Simonsen (1968). In the present study staining activity was obtained, but although individual variation was indicated, the patterns were not clear enough for proper classification of the individuals by the present technique.

Glucose-6-phosphate isomerase (GPI; 5.3.1.9)

Two groups of isoenzymes, probably representing two loci, *GPI-1* and *GPI-2,* were found in the present investigation. Both showed extensive variation, and similar patterns of variations have been described before by Donaghy *et al.* (1995) and Simonsen (1986). Although both isoenzymes could commonly show high activity, the quality of definition was often quite poor for the GPI-2 group which usually gave diffuse bands making any substructure difficult to discern. The diffuse nature of many of the bands could often make reading difficult, however, intraspecific variation was evident at both loci. Both GPI-1 and GPI-2 seemed to follow the pattern of variation of a dimeric protein at a single locus, but only the *GPI-1** genotypes were clear enough for reliable typing of the specimens. Here a pattern of variation was seen that could be explained as segregation of four alleles. They were designated *PGI-1*50, *100, *150* and **170* respectively. The distribution of phenotypic patterns and calculated allele frequencies are presented in Table 2. The phenotype frequency distributions conformed to Hardy-Weinberg expectations.

Glycerol-3-phosphate dehydrogenase (GPD;1.1.1.8)

This enzyme showed up to be monomorphic in most samples of *A. marinus,* and the two different heterozygotes that were seen, occurred too infrequently to be used in population studies.

Isocitrate dehydrogenase (IDHP; 1.1.1.42)

Two, or possibly three, groups of tissue specific isoenzymes were observed; IDHP-1,2 and 3, similarly as described by Donaghy *et al.* (1995). IDHP-1 occurred strongly in the liver but often gave a somewhat diffuse pattern. IDHP-2 was clearly resolved in the muscle as relatively tight bands usually distinctly anodal to IDHP-1. The common allele, *IDHP-2*100,* was displayed monomorphically in the samples from Denmark and Iceland, but two additionale alleles, *IDHP-2*70* and *IDHP-2*130* (called *IDHP-2*118* by Fehervari and Nævdal 1995), was observed at low frequencies in the other samples showing the variation expected of a dimeric protein at a single locus. Faint bands could also generally be seen slightly anodal to IDHP-2 which may either

represent a third locus, *IDHP-3*, or perhaps simply be satellites or some form of shadowing, however, due to the absence of any variability it was impossible to come to any firm conclusions. Frequency distributions of the *IDHP-2** genotypes are shown i Table 3.

Lactate dehydrogenase (LDH; 1.1.1.27)

LDH polymorphism was described by Simonsen (1986), but in the present investigation two invariable LDH bands were found similarly as described by Donaghy *et al.* (1995), and this enzyme does not seem useful in population studies of this species.

Malate dehydrogenase (MDH; 1.1.1.37)

Three groups of isoenzymes, one being polymorphic, were described by Donaghy *et al.* (1995) for this enzyme; and two loci with interlocus heterodimers seem to control the pattern. Simonsen (1996) found no variation in this enzyme. In the present study variation assumed to be the same as described by Donaghy *et al.* (1995) was observed, but due to the low degree of variation, this enzyme was not considered to be infomative for studies on population structure of this species.

Phosphoglucomutase (PGM; 5.4.2.2)

Only a single PGM isoenzyme was found. Strong activity was detectable in both muscle and liver with the same patterns being observed in both tissues, implying the presence of similar systems. Strong activity was also seen under either of the buffer systems, however, TCB buffer was chosen for routine analyses. (The preliminar description (Fehervari and Nævdal 1995) was based upon analyses in the socalled AM-gels, pH 6.1, which gave cathodic migration and accordingly the alleles were given negative designations.) The banding pattern conformed to a model of a single locus encoding a monomeric protein. This locus were found to be highly polymorphic, and five alleles were found, although some very rare. Probably some of these actually consisted of two or three separate very rare alleles, but for population studies they were found to be too difficult to separate. Distributions of phenotypes and calculated observed allele frequencies follow from Table 4. With one exception (sample 5) no significant deviation was found between observed distribution of phenotypes and expected Hardy-Weinberg ditributions of genotypes.

Sorbitol dehydrogenase (SDH; 1.1.1.4)

Weak activity was observed in the muscle extracts of *A.marinus* on the histidin gels only. There appeared to be two invariant loci, however, observable activity was too low for meaningful scoring.

DISCUSSION

The aim of this report was to describe genetic variation within the sandeel species *Ammodytes marinus*, and to present tentative result concerning the structure of this species in the Northeast Atlantic. This is a part of a more extensive project which aim to study the variation between and within sandeel species in the same area.

Tentatively three polymorphic systems were chosen for studies on the population structure of this species. These systems (PGM, PGI-1 and IDHP-2) all displayed clear phenotypes the distributions of which by and large conformed with expected Hardy-Weinberg's distributions. The only significant deviation between observed and expected distribution (PGM, sample 5) can not be given any weight because it is no more than should be expected when conducting a high number of tests. Some other enzymes (PGI-2, AAT and EST) may possibly hold promise for population screening purposes, if these enzyme systems could be examined under more suitable buffer conditions or perhaps through the use of a more powerful technique such as isoelectric focusing. GPD might be used if the material was extensive enough.

Distribution of phenotypes are shown in Tables 2-4. Immediately the variation between samples are surprisingly low implying that *A. marinus* is not or to a low degree structured into different intraspecific groups with their own gene pools. Contingency tests showed that highly significand differences excist among the samples (χ^2=94.5, d.f.=54, P=0.00054), but the greater part of this differences is accounted for by distribution of IDHP types, and the separate χ^2-values together

with calculated genetic distances show that most of the intersample variation is caused by the Icelandic sample. Thus it appears that all other samples are more closely related to each other than they are to the sample from Iceland. However, more heterogeneity is indicated, for instance by the distribution of rare IDHP-2 types, although they are difficult to test by chi square contingency tests due to the low expected numbers. Analyses of a more extensive material could possibly allow more firm conclusions.

These tentative findings seem to be consistent with the geography of the sample sites and the behaviour/biology of the sandeel. Iceland is considerably further away from either of the other sampling sites, which are in closer proximity to each other and separated by relatively shallow water. These distances are probably significant since sandeels have very specific ecological requirements (e.g. sandy substrates and shallower waters), and are not known to make long migratory journeys (Reay 1986), which could effectively limit genetic flow between widely separated areas.

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Table 2. Distribution of PGI -1 types together with calculated allel frequencies for A. marinus.

		Genotype distribution						Allele frequencies			
	Numbers	*50/100*	*100/100*	*100/150*	*100/170*	*150/150*	*150/170*	*50*	*100*	*150*	*170*
1	137	-	74	51	1	10	1	-	0.73	0.263	0.007
2	81	-	59	18	-	3	-	-	0.84	0.154	0.006
3	43	-	22	20	-	1	-	-	0.744	0.256	-
4	161	-	85	60	-	16	-	-	0.714	0.286	-
5	200	1	108	84	-	14	-	0.003	0.776	0.222	-
6	233	2	133	81	2	15	-	0.004	0.753	0.238	0.004
7	60	-	32	21	1	6	-	-	0.717	0.275	0.008

Table 3. Distribution of IDHP -2 types together with calculated allel frequencies for A. marinus.

		Genotype distribution				Allele frequencies		
	Numbers	*70/100*	*100/100*	*100/130*	*130/130*	*70*	*100*	*130*
1	157	2	138	17	-	0.006	0.939	0.054
2	166	-	166	-	-	-	1.000	-
3	50	-	44	6	-	-	0.94	0.06
4	113	-	108	5	-	-	0.978	0.022
5	180	-	170	9	1	-	0.969	0.031
6	70	2	67	1	-	0.014	0.979	0.007
7	60	-	60	-	-	-	1.000	-

Table 4. Distribution of PGM types together with calculated allel frequencies for A. marinus.

		Genotype distribution							Allele frequencies				
	Numbers	*30/100*	*70/100*	*70/150*	*100/100*	*100/150*	*150/150*	*100/170*	*30*	*70*	*100*	*150*	*170*
1	83	2	1	1	88	62	9	2	0.006	0.006	0.736	0.245	0.006
2	171	-	1	1	77	69	23	-	-	0.006	0.655	0.339	-
3	49	-	-	-	24	21	4	-	-	-	0.704	0.296	-
4	150	-	1	-	83	52	14	-	-	0.003	0.73	0.267	-
5	299	-	2	1	175	92	26	3	-	0.005	0.747	0.242	0.008
6	236	-	2	-	126	90	18	-	-	0.004	0.729	0.267	-
7	60	-	1	-	36	16	7	-	-	0.008	0.742	0.25	-