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GENETIC VARIABILITY AT ISOZYME LOCI IN TWO GADOID SPECIES; WHITING (*Merlangius merlangus* L.) AND SAITHE (*Pollachius virens* L.) FROM THE NORWEGIAN COAST.

by

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ABSTRACT

Genetic variation in whiting (*Merlangius merlangus*) and saithe (*Pollachius virens*) was investigated by starch gel electrophoresis of enzymes in tissue extracts. 94 specimens of whiting and 96 specimens of saithe were collected by trawl in two fjords in mid-Norway; Trondheimsfjorden and Romsdalsfjorden. A total of 20 tissue enzymes revealed 10 reliably scored loci in both species. Variant alleles (99% criterion) were found at five loci in each of the species (*G3PDH-2**, *IDHP-1**, *PGI-1**, *PGI-2**, *PGM** in whiting and *G3PDH-2**, *IDHP**, *LDH-2**, *PGI-1**, *PGI-2** in saithe). The frequency of polymorphic loci is then P = 0.50 for both. In whiting the average heterozygosity per locus was H = 0.164, the effective number of alleles per locus varied from 1.01 (*LDH-1**, *LDH-3**) to 3.26 (*PGI-2**) with a mean of 1.39. In saithe the average heterozygosity per locus was H = 0.036, the effective number of alleles per locus varied from 1.02 (*PGI-1**, *PGI-2**) to 1.17 (*G3PDH-2**) with a mean of 1.04. Details of electrophoretic conditions, staining procedures, and tissue manifestation of the loci are described.

key words: whiting; *Merlangius merlangus*; saithe; *Pollachius virens*; isozymes; fisheries management; population genetics; starch gel electrophoresis.

INTRODUCTION

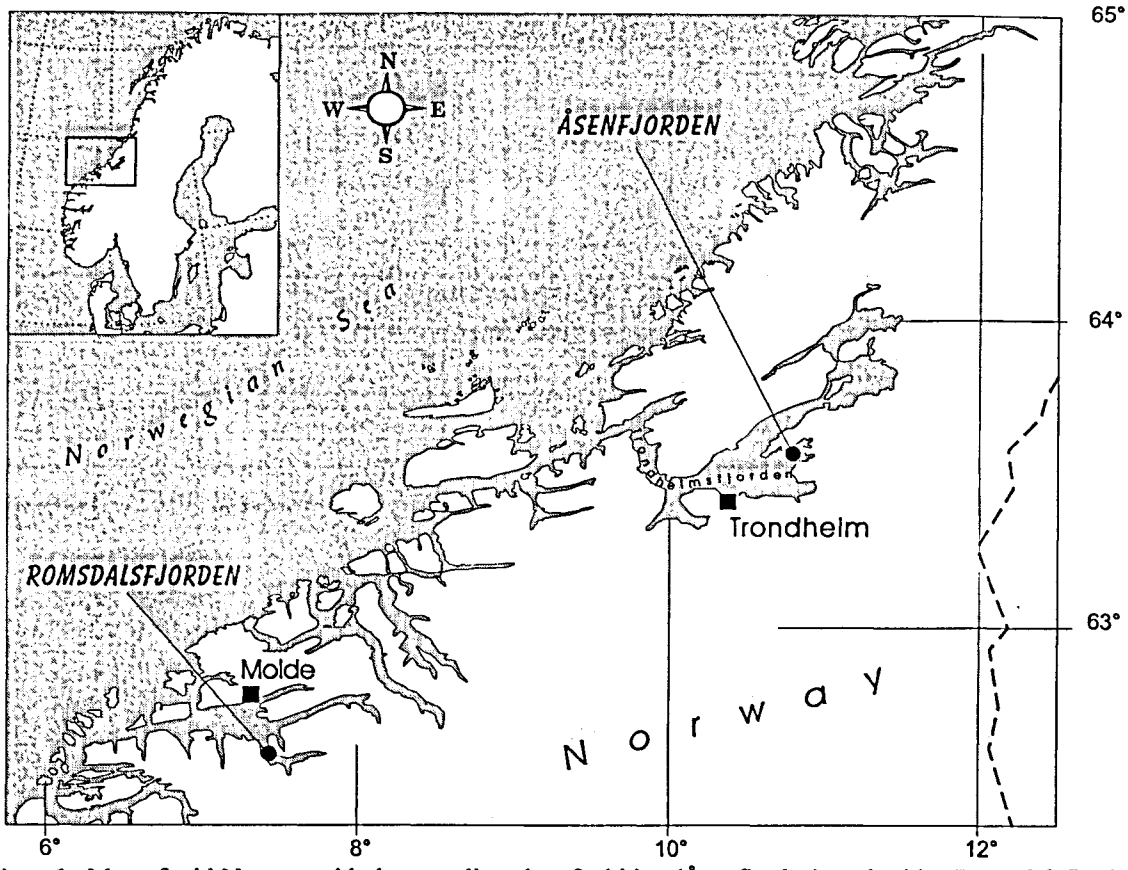
The saithe (*Pollachius virens* Linneaus, 1758) is an Atlantic amphiboreal gadoid species. Compared to most cod-like fishes, saithe spends relatively less time on bottom and more time moving freely in the water column. Studies indicate a broad overall depth range of 30-370 m and a preferred depth range of 110-180 m (Scott and Scott 1988). Its distribution in the western North Atlantic is from off southern Labrador at Sandwich Bay, around Newfoundland and the Grand Bank, Gulf of St Lawrence and southward to about Cape Hatteras. In the eastern North Atlantic it is found from Biscay to Greenland, and eastward from the North Sea along the Norwegian coast to Kola and more rarely at Spitsbergen (Scott and Scott 1988). Important East Atlantic spawning grounds are found off the Norwegian west-coast, in the northern North Sea, at Shetland, Faroe Islands and Iceland (Schmidt 1909). Spawning takes place mainly in February-March at 150-200 m deep. In the West Atlantic spawning is reported from september to april. Eggs and larvae are pelagic. Saithe usually reach sexual maturity at an age of 3-6 years, and is then 40 - 60 cm (Svetovidov 1948; Scott and Scott 1988). It prefers temperatures of 5-8°C and a salinity near 35‰ but can withstand temperatures as low as 0°C. Next to cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) it has traditionally been one of the most commercially important species in the north East Atlantic for centuries. Its abundance in the north West Atlantic has increased significantly in the last decades (Scott and Scott 1988).

Whiting (*Merlangius merlangus* Linnaeus, 1758) is a gadoid which in European Atlantic waters is distributed from northern Norway and Iceland in the north to Biscay in the south, with the main stock in the North Sea. A subspecies (*M. m. euxinus*) is found in the Black Sea and less commonly in the eastern Mediterranean. Whiting is predominantly a shallow water species, with the major part of the commercial catch being taken between 25-150 m. While the life span can be up to seven years and the length can be up to 70 cm it rarely reach lengths greater than 30-40 cm. It is thus typically smaller than related gadoids such as cod, haddock and saithe. It reaches maturity from two years old and spawns from January to June, depending on latitude. Important spawning grounds in the East Atlantic are found in shallow waters (<100 m) in the North Sea and Skagerrak (Knutsen 1968). Whiting have a longer pelagic phase than most other common gadoids in the North Sea, resulting in a wide geographic dispersion during the young stages. Due to economically important fisheries, whiting is of special interest to the countries around the North Sea, especially Great Britain, France and Holland, who take most of the annual landings (about 150 000 tons) (Knijn et al. 1993).

MATERIALS AND METHODS

Sample collection

94 specimens of whiting and 96 of saithe were collected by trawl (RV "Michael Sars; October 1994) Åsenfjorden and Romsdalsfjorden, mid-Norway (Fig. 1 and Table 1). The cruise was part of a coastal resource study program run by The Norwegian Institute of Fisheries and Aquaculture, Ltd., Tromsø, Norway, which also provided technical assistance during sampling. Tissue samples (muscle and liver) were cut out and frozen (at -20°C) in individually numbered plastic bags immediately after catch. After two weeks they were transfered to -82°C storage and kept at this temperature for two years before analyses. Biological data (length, weight, sex) were recorded for each specimen, and otoliths collected for age determination.



Figur 1. Map of mid-Norway with the sampling site of whiting (Åsenfjorden), and saithe (Romsdalsfjorden).

Tissue extract preparation

Muscle, liver, and heart tissues were separately homogenized in equal amounts of distilled water and centrifuged (10 min at 10 000 g, 4°C). The supernatant was used for electrophoresis.

Electrophoresis

Two buffer systems were used; the discontinuous system by Ridgway *et al.* (1970) (RW) and the continuous system by Clayton and Tretiak (1972) (AC). 10 mm thick starch gels (11%) were moulded directly onto ceramic cooling plates. Tissue extract samples were applied to the gel in filter paper (1x12 mm) inserted along a slot cut 4 cm from the cathodic electrode. The filter papers were removed after 15 minutes run at 0.08 W/cm^2, whereafter the wattage was raised to 0.10 W/cm^2. Electrophoresis was completed after 2 hrs with the RW system and 4 to 4.5 hrs with the AC system.

Staining

After electrophoresis, the gel was sliced into one mm slices which were incubated in the respective staining solutions and left in the dark (37°C) until sufficiently strong bands had developed. In general, the staining recipes followed Hillis and Morris (1990) with the following modifications: except for SOD, the staining solutions were modified by the replacement of phenazine methosulphate (PMS) with phenazine ethosulphate (PES), and the pH in the staining solutions was raised to 9.0 when staining for dehydrogenases (Mork 1990).

Table 1. Saithe and whiting sample information. N = Number of individual analysed

Sample	Species	Station code	Position	Research Vessel	N	Date	Trawl type and mesh width
Åsenfjorden	Whiting	628	62°33' N 10°45' E	Michael Sars	94	09.10.94	Bottom trawl (35 mm)*
Romsdalsfjorden	Saithe	642	62°35' N 7°25' E	Michael Sars	96	12.10.94	Bottom trawl (35 mm)*

* With a 10 mm mesh insert in the cod-end.

Interpretation of banding patterns

The genetic interpretation of banding patterns followed the principles outlined by Allendorf *et al.* (1977). Nomenclature of loci, genotypes and alleles accords to Shaklee *et al.* (1990). If more than one buffersystem are listed in Table 2 and 4, the first one was used in this study, but both are usable.

Enzymes stained for	E.C. number	Abbreviation
Alcohol dehydrogenase	E.C. 1.1.1.1	ADH
Aspartate aminotransferase	E.C. 2.6.1.1	AAT
Aconitase	E.C. 4.2.1.3	ACO
Adenylate kinase	E.C. 2.7.4.2	AK
Creatine kinase	E.C. 2.7.3.2	CK
Fumarate hydratase	E.C. 4.2.1.2	FUMH
Glyceraldehyde 3-phosphate dehydrogenase	E.C. 1.2.1.12	GAPDH
Glucose-6-phosphate dehydrogenase	E.C. 1.1.1.49	G6PDH
Glucose-6-phosphate isomerase	E.C. 5.3.1.9	PGI (GPI)
Glutathione reductase	E.C. 1.6.4.2	GR
Glycerol-3-phosphate dehydrogenase	E.C. 1.1.1.8	G3PDH
Hexokinase	E.C. 2.7.1.1	HK
L-Iditol dehydrogenase	E.C. 1.1.1.14	IDDH (SDH)
Isocitrate dehydrogenase	E.C. 1.1.1.42	IDHP
L-Lactate dehydrogenase	E.C. 1.1.1.27	LDH
Malate dehydrogenase	E.C. 1.1.1.37	MDH
Malate dehydrogenase phosphate	E.C. 1.1.1.40	MDHP (Me)
Phosphoglucomutase	E.C. 5.4.2.2	PGM
Superoxide dismutase	E.C. 1.15.1.1	SOD
Xanthine dehydrogenase	E.C. 1.1.1.204	XDH

RESULTS

WHITING AND SAITHE:

The main results from the genetic analysis are compiled in Table 2 (Whiting) and 4 (Saithe), while allele frequencies, effective numbers of alleles (n_e), and observed and expected heterozygosities (H_{obs} and H_{exp}) for whiting and saithe are compiled in Table 3 and 5, respectively.

Enzyme activity, tissue manifestations of loci, and preferred electrophoretic conditions were very similar in these two species (and also similar to those for haddock (Forthun and Mork 1997)).

In both species, 11 enzymes did not show patterns distinct enough to permit any conclusions about their genetic control (ADH, ACO, AK, CK, FUMH, GAPDH, G6PDH, GR, HK, IDDH and XDH), and are not included in the tables. Background lactate dehydrogenase activity was observed in many of these: ADH, FUMH, GAPDH, IDDH, and XDH. The observed banding patterns and tissue expression for the other enzymes were assigned to loci which we here treat in three groups: monomorphic, polymorphic (99% criterion), and uninterpreted.

WHITING:

Of the 10 loci interpreted in this study, five were polymorphic by the 99% criterion (*G3PDH-2*, IDHP-1*, PGI-1*, PGI-2*, PGM**) ($P_{0.99}$ = 0.50), while three were polymorphic by the 95% criterion (*IDHP-1*, PGI-2*, PGM**) ($P_{0.95}$ = 0.30).

Monomorphic loci (*LDH-1*, LDH-2*, LDH-3*, MDH-2*, SOD**)

*LDH-1** and *LDH-3** showed one variant each (*LDH-1*-420* and *LDH-3*115*). *LDH-2**, *MDH-2** and *SOD** did not show any variants. *LDH-1** sometimes showed poor resolution in some individuals, but a rerun solved the problem. *LDH-3** showed very good resolution but the activity was low. However after staining for 2-3 hrs at 37°C the bands were visible.

Polymorphic loci (*G3PDH-2*, IDHP-2*, PGI-1*, PGI-2*, PGM**)

*PGI-2** showed good staining with both buffersystem, but several alleles were not detected with the RW system. The AC buffersystem gave anodic satelittes which complicated interpretation, but a prolonged run of the gel (at least 4 hrs) gave interpretable zymograms. There appeared to be several alleles intermediate between the **100* and **177* allele, but they were all difficult to score consistently. In the data presented these alleles are therfore pooled and named **144* after the most frequent one. They constitute about 20% of the alleles and are not visible with the RW system, where they can not be distinguished from the homozygote of *PGI-2*100*. *G3PDH-2*, IDHP-2*, PGI-1**, and *PGM** are all analysed with RW, which gave strong bands and good resolution in all of them.

Uninterpreted loci (*AAT-1*, AAT-2*, G3PDH-1*, IDHP-1*, MDH-1*, MDHP**)

*AAT-1** and *AAT-2** appear to be represented by a single invariant band. However poor resolution and somewhat low activity prevent them for use in this study. *G3PDH-1* showed good resolution but very poor activity. However, two variant alleles were found in two out of about 40 scored individuals. *IDHP-2** and *MDH-1** were expressed with the AC buffersystem only. Unreliable resolution and weak bands make them less useful in population surveys. *MDHP** did show good resolution, but the activity was variable. It seems to be represented by a single invariant locus in the tissue used here.

Table 2. Whiting enzymes which gave specific staining reaction. Enzymes stained for, locus designation, tissues with best activity and resolution, preferred buffer system, alleles found. Abbr.: M = muscle; L = liver. See text for further explanation.

Enzyme	Locus	Best tissue	Buffer system	Alleles	Comments
AAT	*AAT-1**	M	RW	-	Catodic migration
	*AAT-2**	L	RW	-	Anodic migration
G3PDH	*G3PDH-1**	L	RW	*25, 100, 200*	Low and variable activity
	*G3PDH-2**	M	RW	*79, 91, 100*	
IDHP	*IDHP-1**	L	RW	*80, 100, 132*	
	*IDHP-2**	M	AC	-	Insufficient resolution
LDH	*LDH-1**	L	AC, RW	*-420**, *-100*	
	*LDH-2**	M	RW, AC	*100*	
	*LDH-3**	M	RW, AC	*87, 100*	Low activity
MDH	*MDH-1**	M	AC	-	Low activity
	*MDH-2**	L	AC	*100*	
MDHP	*MDHP**	M	RW	-	Low and variable activity
PGI	*PGI-1**	M	RW	*70, 100*	
	*PGI-2**	L	AC	*53, 68, 100, 144***, *177, 203, 207, 229*	
PGM	*PGM**	L	RW	*100, 116*	
SOD	*SOD**	L	RW, AC	*100*	Low activity

*A minus "-" before the allele indicate cathodic mobility. **Represents more than one allele, see text.

Table 3. Whiting allele frequencies, effectiv number of allels (n_e), and observed and expected heterozygosity at each locus.

Locus	Allele frequency								n_e	H_{obs}	H_{exp}
	100	*91*	*79*								
*G3PDH-2**	0.973	0.011	0.016						1.06	0.053	0.052
	100	*80*	*132*								
*IDHP-1**	0.787	0.197	0.016						1.82	0.362	0.341
	-100	*-420*									
*LDH-1**	0.995	0.005							1.01	0.011	0.011
	100										
*LDH-2**	1.000								1	0	0
	100	*87*									
*LDH-3**	0.995	0.005							1.01	0.011	0.011
	100										
*MDH-2**	1.000								1	0	0
	100	*70*									
*PGI-1**	0.984	0.016							1.03	0.032	0.031
	100	*144**	*177*	*203*	*207*	*229*	*53*	*68*			
*PGI-2**	0.399	0.197	0.324	0.016	0.005	0.021	0.011	0.027	3.26	0.681	0.694
	100	*116*									
*PGM**	0.537	0.463							1.99	0.543	0.498
	100										
*SOD**	1.000								1	0	0

SAITHE:

Electrophoretic analysis of a total of 20 tissue enzymes gave 10 readable loci. In five of these (*G3PDH-2**, *IDHP**, *LDH-2**, *PGI-1**, *PGI-2**) there were found variants in frequencies satisfying the 99% criterion ($P_{0.99} = 0.50$) and two of these (*G3PDH-2**, *LDH-2**) were inside the 95% criterion limits ($P_{0.95} = 0.20$). *LDH-1**, *LDH-3**, *MDH-2**, *PGM**, *SOD** did not show any variation.

Monomorphic loci (*LDH-1**, *LDH-3**, *MDH-2**, *PGM**, *SOD**)

Except for *MDH-2**, which was expressed in AC only, all of these loci were expressed in both buffersystems with good staining intensity and band distinctness.

Polymorphic loci (*G3PDH-2**, *IDHP**, *LDH-2**, *PGI-1**, *PGI-2**)

*G3PDH-2** and *IDHP** were both staind for with RW, which gave good resolution and high enzyme activity. *LDH-2**, *PGI-1** and *PGI-2** were well expressed in both RW and AC.

Uninterpreted loci (*AAT-1**, *AAT-2**, *G3PDH-1**, *MDH-1**, *MDHP**)

Aspartate aminotransferase seemed to be represented by two invariant loci, here designated *AAT-1** and *AAT-2**. Variable activity and poor resolution made it difficult to use them in routine analyses. *G3PDH-1** and *MDHP** were expressed in RW only. Both showed good resolution but variable activity. However, no variation seemed to be expressed in the *MDHP**. As for *MDH-2* MDH-1** was expressed in AC, but only as weak shadows.

Table 4. Saithe enzymes which gave specific staining reaction. Enzymes stained for, locus designation, tissues with best activity and resolution, preferred buffer system, alleles found. Abbr.: M = muscle; L = liver. See text for further explanation.

Enzyme	Locus	Best tissue	Buffer system	Alleles	Comments
AAT	*AAT-1**	M	RW	-	Catodic migration
	*AAT-2**	L	RW	-	Anodic migration
G3PDH	*G3PDH-1**	L	RW	-	Low and variable activity
	*G3PDH-2**	M	RW	*90, 100*	
IDHP	*IDHP**	L	RW, AC	*100, 125*	
LDH	*LDH-1**	L	AC, RW	*-100**	
	*LDH-2**	M	RW, AC	*100, 128*	
	*LDH-3**	M	RW, AC	*100*	Low activity
MDH	*MDH-1**	M	AC	-	Low activity
	*MDH-2**	L	AC	*100*	
MDHP	*MDHP**	M	RW	-	Low and variable activity
PGI	*PGI-1**	M	RW	*69, 100, 125*	
	*PGI-2**	L	AC	*90, 100, 109*	
PGM	*PGM**	L	RW	*100*	
SOD	*SOD**	L	RW, AC	*100*	Low activity

*A minus "-" before the allele indicate cathodic mobility.

Tabell 5. Saithe allele frequencies, effective number of alleles (n_e), and observed and expected heterozygosity at each locus.

Locus	Allele frequency			n_e	H_{obs}	H_{exp}
	100	*90*				
*G3PDH-2**	0.922	0.078		1.17	0.156	0.144
	100	*125*				
*IDHP**	0.979	0.021		1.04	0.042	0.041
	-100					
*LDH-1**	1.000			1	0	0
	100	*128*				
*LDH-2**	0.927	0.073		1.16	0.146	0.135
	100					
*LDH-3**	1.000			1	0	0
	100					
*MDH-2**	1.000			1	0	0
	100	*69*	*125*			
*PGI-1**	0.990	0.005	0.005	1.02	0.021	0.021
	100	*109*	*90*			
*PGI-2**	0.990	0.005	0.005	1.02	0.021	0.021
	100					
*PGM**	1.000			1	0	0
	100					
*SOD**	1.000			1	0	0

DISCUSSION

Studies of genetic variability between and within populations should be based on a random sample of the genome. This requires a sample which is both diverse with regard to biological function of loci, and unbiased with regard to monomorphism versus polymorphism. Here a total of 20 enzymes, approximately 25-30 loci, were examined in the search for usable markers in population genetic surveys. The examination revealed a minimum of 16 loci at whiting and 15 loci at saithe. 10 were considered as usable in both species. Some adjustments in methodology could make *AAT-1**, *AAT-2** and *MDHP** usable as well.

The quantitative estimates of the amount of genetic variability in this study were based on the 10 loci considered potentially useful in population genetic surveys. The frequency of polymorphic loci in the present material was $P_{0.95} = 0.30$ and $P_{0.99} = 0.50$ in whiting and $P_{0.95} = 0.20$ and $P_{0.99} = 0.50$ in saithe. The expected average heterozygosity per locus was estimated to $H = 0.164$ in whiting, but considerably lower ($H = 0.036$) in saithe (Table 6).

Table 6. Amount of polymorphic loci and mean heterozygosity pr. locus in whiting and saithe.

	Polymorphism		Heterozygosity	
	$P_{0.95}$	$P_{0.99}$	H	Comments:
Whiting:	0.30	0.50	0.164	10 loci
Saithe:	0.20	0.50	0.036	10 loci

Compared with the variability estimates for cod ($P_{0.95}$ = 0.20, $P_{0.99}$ = 0.30 and H = 0.082) (Mork *et al.* 1982), which were based on 30 loci from cod sampled in a fjord in mid-Norway, whiting showed about twice the heterozygosity of the cod, and saithe only about the half. Nevo (1978) estimated average values for the frequency of polymorphic loci and average heterozygosity for bony fishes in general to be 0.15 and 0.05, respectively.

In whiting, *IDHP-1**, *PGI-2** and *PGM** appears to have the largest potentials as genetic markers. In saithe *G3PDH-2** and *LDH-2**, with frequency of the most common allele estimated to be 0.92 and 0.93 respectively, were the most promising loci.

Knowledge about genetic population structure in order to identify biologically meaningfull management units is essential for the long term rational management of marine resources. Population genetic studies using electrophoretic markers can aid in achieving this. For the species under study here, the majority of the genetic markers presented in this paper have not previously been described in the open literature. They may provide useful tools for future studies of the genetic structure of these species.

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