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ESTERASE VARIATION IN HERRING STUDIED BY ISOELECTRIC FOCUSING

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ABSTRACT

Few suitable genetic characteristics for studying herring population structure are available, and the main aim of the study reported here has been to reveal traits which display useful genetic variations for application on more extensive population studies of herring in Northeast Atlantic waters. Esterase is often found to be highly variable among fishes, but often the phenotypes are difficult to discern and thus the genetic background for the observed variation is difficult to reveal. In the present report isoelectric focusing of blood esterases has been used as an analytic tool for revealing and describing variations and their genetic background. Material has been collected from coastal and offshore Norwegian waters, and more than 1500 specimens of 24 samples have been analysed at two pH ranges (3.5-9.5 and 4.0-6.5). Four groups of esterase components were found, each probably representing at least one polymorphic locus. Two of these groups were clear enough for proper classification of the individuals, and at least one seemed useful for further studies of herring population structure. Clear variations were seen in phenotype distributions between samples, and especially the samples of autumn spawning herring deviated from the bulk of the spring spawners.

Key words: esterase, herring, isoelectric focusing, population genetics

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INTRODUCTION

Electrophoretic studies of Atlantic herring, *Clupea harengus* L, esterase have been conducted by several authors (Nævdal 1969, Simonarson & Watts 1969, Ridgway & al. 1970). Similarly Utter (1972) and Grant (1981) described electrophoretic variation in Pacific herring. Evidently, the aims have been to reveal and describe traits which could be used in population genetic studies of this species. As far as can be judged the studies have been partly successful, and to some degree esterase polymorphism has been used for studies on population structure. However, esterases have never been used in any extensive study on Atlantic herring populations, possibly because the observed variations have been complex and require high technical resolution of the multiple components to be used for reliable classification. Generally, allozyme studies have revealed a high level of genetic variation in herring, but with one exception (Jørstad & al. 1994), most of these variations are distributed among individuals within populations, not between populations (Anderson & al. 1981, Jørstad & al. 1990). For this reason there is a clear need for additional traits to be used as tools for population structure studies in herring. Different types of DNA-anlyses are being developed (Dahle & Eriksen 1992, Jørstad & al. 1994), but also variation in functional proteins will be valuable. Therefore this study was undertaken to evalute esterase variation as population genetic tool based upon improved analytic technique.

MATERIAL AND METHODS

Material for the present study were collected in Norwegian coastal and offshore waters during Spring and Fall 1996. An overview of the material is given in Table 1 showing sampling locality, numbers within each sample and characteristics of each sample (mean age, size etc.). The material was collected to cover the different fish sizes, and likewise different potential populations should be represented. The same material and partly the same gels were used to study hemoglobin variation because preliminar analyses had shown that the two groups of proteins esterase components were well separated from each other on the same gels. Hemoglobins are not further treated here. To look for possible *in vitro* changes part of the material was analysed both fresh and frozen and after different storage time in the refrigerator.

Blood was collected from freshly killed herring with a syringe from the heart or by cutting the tail (small fishes). The blood was centrifuged, plasma pipetted off and the cells were lysed by adding destilled water or by ultrasound teatment. Because herring red blood cells are very easily ruptured, it showed up to be very difficult to have plasma free of hemoglobins for the majority of samples. Therefore, after initial analyses of both serum and hemolysate, it was decided to base the analyses on washed lysed blood cells.

The esterase components were analysed by isoelectric focusing carried out according to the instruction manuals of the MultiphorII system (LKB) with precast slab gels (Ampholine PAG©plates, Pharmacia), pH 3.5-9.5 and 4.0-6.5. The samples were applicated on the middle of the gels. The gels were stained for esterase activity using the solution described by Harris & Hopkinson (1976), photographed by transmitted light, and stored at 4°C.

RESULTS

Isoelectric focusing gave high resolution of the esterase as well as the hemoglobin components on the same gel. The gels with the broadest pH range were also stained for hemoglobins and used for descriptions of hemoglobin variations (Nævdal & al. manuscript). Very little overlapping was found between esterase and hemoglobin components, the first ones displaying anodal and the second ones cathodal migrations. The esterase components were generally better resolved by using the gels with the narrower pH range, and the descriptions below are based on the results obtained with these gels and supported with corresponding results using the gels with the wide pH-range. A picture of a gel is shown in Figure 1.

Multiple freezing and thawing did not influence the results except that the components became weaker and consequently more difficult to read after repeated freezing. Plasma and cells contained the same esterase components although not in the same proportions. Because of the difficulties mentioned above concerning partly lysis of most samples, the description is based on results from analysis of cell hemolysate.

The esterase components were separated into four groups, called EST-1, EST-2, EST-3 and EST-4 in order of increasing anodic mobility, each of which are supposed to be controlled by at least one locus. This is in accordance with the descriptions of Ridgway & al. (1970) and Grant (1981). No variation which could be connected to fish age was indicate.

The EST-1 group was found to be stronger in plasma than in the cells. It consist of one or two components indicating a multiallelic system of monomeric proteins. At least five bands were recognized, but due to their weakness and their similarity with respect to migrating distance (isoelectric point), the bands and consequently their putative controlling alleles, were lumped together into two groups. The alleles are tentatively called *EST-1*70* and *EST-1*100* . With this simplification the specimens of most samples could be grouped with respect to EST-1 phenotypes when both types of gels were compared. The bands were better separated in the narrow gel, but the components became weaker compared to the broad gel. Distributions of phenotypes are given in Table 2, together with frequencies of putative controlling genes. However, compared to expected Hardy-Weinberg distribution (not shown) the fit was not very good, and thus the mode of inheritance put forward here does not seem to be correct or at least imperfect.

The EST-2 components also were found both in plasma and cells, often somewhat stonger in plasma. Much variation was indicated although not very clear and further complicated by variable strength of the components. At the moment it was therefore found impossible to group the components and reveal the genetic background of the variation.

The EST-3 group is composed of one or two strong and several weak bands. This variation may be explained as homozygotes and heterozygotes in a polyallel system of monomeric proteins, but the presence of weak bands may complicate the classification. Comparison of the patterns from the narrow and the broad gels improved the reliability of the classification, and practically all specimens could be classified. Tentatively the putative alleles were named *EST-3*70, *80, *100 and *120.* Distribution of phenotypes in all samples are shown in Table 2 together with frequencies of the putative controlling genes. When comparing to expected Hardy-Weinberg distribution (data not shown) the fit was found to be reasonably good indicating that the mode of inheritance put forward here is correct.

EST-4 is a group of weak components. Variations are indicated also here, but the components are two weak to be further utilized and are therefore omitted here.

DISCUSSION

Because the technique used here is different compared to the earlier analytic techniques used in esterase analyses, direct comparisons of the results are difficult. However, as pointed out among others by Ridgway & al. (1970) evidently at least parts of the esterase components are tissue specific. The main argument for using blood is that it is easy to sample and the esterase patterns seem less complicated than in other tissues. The minor differences between plasma and cells also seem to make it justified to use whole blood for routine investigations. The fact that repeated thawing and freezing did not effect the results faciliates routine investigations because direct comparisons on the same gel of samples collected at different times improve the reliability of the typings.

The extensive variation in blood esterase is tentatively explained as being controlled by four gene loci which all show individual variation. For further use in population studies it is an absolute requirement that the results are repeatable, and that the genetic background of the variations are understood. In this case the groups EST-1 and EST-3 were the only ones which seemed to fulfill the first requirement. Although the patterns of EST-1 in most cases are repeatable, the weakness of the components requires a very high technical quality of the gels to obtain unambigous classification of the specimens. The components often were of uneven strength, and therefore one of the components in a heterozygotous pattern could easily be overlooked. This may be the main reason for the heterozygote deficiency observed in most samples compared to expected Hardy-Weinberg distributions. Although freezing and thawing by itseld did not seem to effect the patterns, the components became weaker and thus more difficult to read, even after the

simplification presented in Table 2. For this reason we still hesitate to use the EST-1 variation as population parameters and consequently draw conclusion about the herring structure based upon this system.

On the other hand EST-3 seems promising both concerning repeatability, genetic control and intrapopulation variation. As expected the samples from inshore waters deviated from oceanic ones represented by Norwegian Spring spawning herring as well as adolescent and adult herring from the North sea. At least one of the inshore samples (no 12) represents autumn spawners which deviated significantly from the bulk of Spring spawners. The present material, however, is good enough for preliminar studies aimed to reveal population parameter traits, but not structured enough for drawing conclusions about gene pools. However, $\chi 2$-tests revealed a clear heterogeneity among the samples, and most of this heterogeneity is caused by the deviating inshore samples. Largely allozymes have not shown enough interpopulation variations in herring to make them useful for studies on population structure (except in fjords in Northern Norway). DNA traits (mtDNA, mini- and microsatellites) seem promising for studying the genetic structure of herring in areas where the stock structure still is incompletely understood, but also the blood esterase EST-5 seems useful for such purposes.

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Table 1. Account of herring sampled for analyses of variations in esterase components in 1996. NSS: Norwegian spring spawning herring

Sample no	Date of sampling	Locality	Numbers	Age and size characteristics
1	11 Jan	Bjorøy, Hordaland	60	spawning coastal herring
2	3 March	62°26'N/5°22'E	99	spawning NSS
3	5 "	63°36'N/5°02'E	90	"
4	6 "	62°05'N/4°58'E	45	"
5	6 "	61°59'N/4°02'E	50	"
6	6 "	61°54'N/4°32'E	60	"
7	6 "	61°46'N/4°32'E	95	"
8	6 "	Bjorøy, Hordaland	50	spawning coastal herring
9	8 "	59°15'N/5°04'E	100	spawning NSS
10	14 "	61°43'N/4°29'E	96	"
11	27 "	Karmøy, Rogaland	96	" (?)
12	26 Sept	Herdlafjorden, Hordaland	96	adult autumn spawner
13	27 "	Bjorøy, Hordaland	90	I-group
14	21 Oct	57°41'N/5°27'E	25	I-group
15	23 Oct	57°17'N/1°46'E	25	adult, < 34 cm
16	26 "	61°12'N/0°15'E	25	adult, < 26 cm
17	30 "	59°47'N/0°28'E	50	different age groups 34 cm
18	3 "	57°35'N/9°14'E	87	0- and I-group
19	5 "	59°03'N/11°10'E	176	0- and I-group
20	6 "	59°01'N/9°47'E	50	10-30 cm
21	10 "	56°16'N/1°32'E	31	I-III-group
22	13 "	56°40'N/6°29'E	20	0-II-group
23	24 "	Bjorøy, Hordaland	20	II-group (?)

Table 2. Distribution of esterase types in herring together with frequencies of putative genes.

		EST-1					EST-3								
		Distribution			Gene frequencies		Distribution					Gene frequencies			
nr.	*N*	*70/70*	*70/100*	*100/100*	*70*	*100*	*70/100*	*80/80*	*80/100*	*100/100*	*100/120*	*70*	*80*	*100*	*120*
1	60	-	18	30	0.19	0.81	-	-	11	45	-	-	0.10	0.90	-
2	99	5	23	60	0.19	0.81	-	-	1	95	-	-	0.01	0.99	-
3	90	not readable					-	-	1	67	1	-	0.01	0.98	0.01
4	45	2	9	26	0.18	0.82	-	-	1	41	-	-	0.01	0.99	-
5	50	not readable					-	-	-	40	1	-	-	0.99	0.01
6	60	3	10	37	0.16	0.84	-	-	1	59	-	-	0.01	0.99	-
7	95	not readable					-	-	8	35	1	-	0.09	0.90	0.01
8	50	1	7	40	0.09	0.91	1	-	23	23	-	0.01	0.24	0.75	-
9	100	9	15	76	0.16	0.84	2	-	5	93	-	0.01	0.03	0.96	-
10	88	3	16	54	0.15	0.85	-	-	7	77	4	-	0.04	0.94	0.02
11	96	6	11	66	0.14	0.86	-	2	11	86	-	-	0.08	0.92	-
12	96	6	13	56	0.17	0.83	1	14	29	49	-	0.01	0.31	0.68	-
13	90	not readable					2	2	16	68	-	0.01	0.11	0.88	-
14	25	1	4	15	0.15	0.85	-	-	1	24	-	-	0.02	0.98	-
15	25	not readable					-	-	1	24	-	-	0.02	0.98	-
16	25	- " -					-	-	-	25	-	-	-	1.00	-
17	50	not readable					2	-	8	38	2	0.02	0.08	0.88	0.02
18	87	-	16	43	0.14	0.86	1	-	5	80	1	0.01	0.03	0.96	0.01
19	176	4	22	79	0.14	0.86	3	-	5	168	-	0.01	0.01	0.98	-
20	33	8	8	10	0.46	0.54	-	-	1	32	-	-	0.02	0.98	-
21	31	3	11	24	0.22	0.78	2	-	7	47	-	0.02	0.06	0.92	-
22	20	not readable					-	-	5	15	-	-	0.13	0.87	-
23	21	1	20	-	0.02	0.98	3	-	10	7	-	0.08	0.25	0.67	-

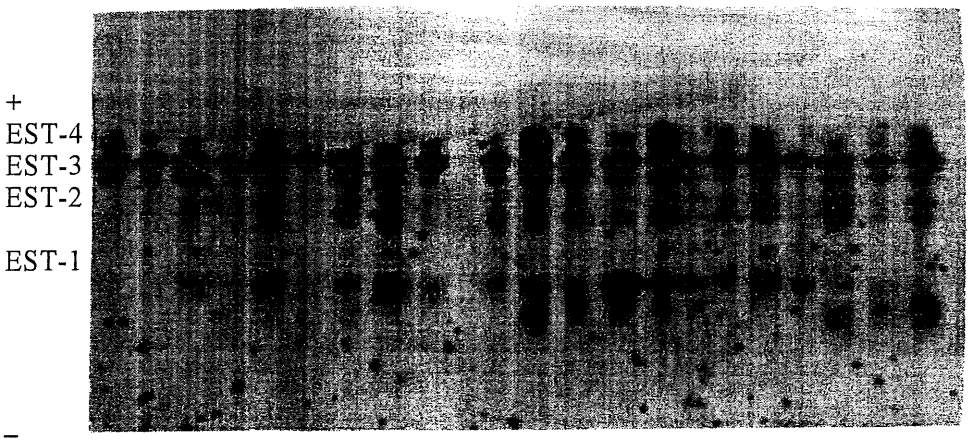

Figure 1. Blood esterase in herring. Photo of an isoelectric focusing gel, pH 4.0-6.5, after staining