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# A STUDY OF GENETIC VARIATION IN NORTHEAST ATLANTIC HARP SEALS (*PAGOPHILUS GROENLANDICUS*)

By

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## ABSTRACT

16 enzymes were examined for genetic studies of the harp seal population structure in the Northeast Atlantic. Muscle and liver samples were collected in the West Ice off Jan Mayen and in the Eastern Barents Sea in 1987, 1989 and 1990. The samples were analyzed by starch gel electrophoresis and isoelectric focusing.

Among 25 resolved loci, 12 were found to be variable. By isoelectric focusing, muscle tissue was found to express five polymorphic esterase systems, but due to technical difficulties, only EST-3 was consistently resolved. EST-3 and another three systems (AAT-2, ALP-2 and LDH-1) were polymorphic at the 95% criterion and were employed in comparative analysis. Another four loci were variable, but alternative alleles in these loci were rare. Most variable loci were exclusively resolved with isoelectric focusing.

No significant variation among samples were found, although the sample collected off Jan Mayen in 1989 derived from the rest of the samples in phenotype distributions in three of the four polymorphic systems.

Gel: 9,6 g L-Histidine-HCl in 10 l dest. water. Adjusted to pH 7,0 with NaOH

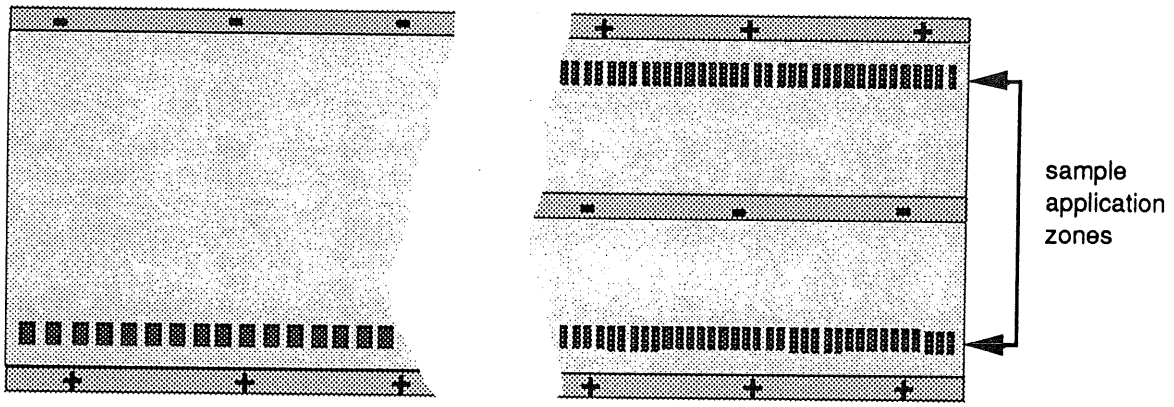
b) Bridge: 4,2 g. citric acid in 10 l dest. water. Adjusted to pH 6,1 with N(3-aminopropyl)morpholine.

Gel: 1 in 20 dilution of bridge buffer.

A potential of 13 volts/cm was applied across the gel for two to three hours. (Principles and analytical setups for s.g.e. are described in Brewer (1970) and Smith (1976)).

IFPAG is a method where samples are introduced to a thin layer polyacrylamide gel in which a pH gradient has been established. The proteins migrate in the gel until they have reached the point in the pH gradient equivalent to their isoelectric points (pI). Principles for isoelectric focusing are further described in Leaback and Wrigley (1976), and practical information is obtained from Pharmacia LBK Biotechnology, Uppsala, Sweden. The information on IFPAG setups suggests a maximum of 52 individuals analyzed for one enzyme system per gel. Yet, for consistently resolved systems, two different strategies were useful in order to handle a larger number of samples: Firstly, a reduction of the application pieces allowed 70-80 samples per gel (fig. 1). Secondly, this number could be doubled by introduction of a second anode which was employed in order to create two identical pH ranges on the same gel (fig. 1).

Fig. 1. An alternative method for IFPAG analytical setup. The figure contrasts the traditional setup and adapted present setup: On the right side, application piece size is reduced in order to increase the number of samples on each gel. This capacity is again doubled by introducing a third electrode, creating two pH ranges on each gel.




Voltage, current and power settings were not modified. In addition to the increased sample capacity, each gel could be stained for different enzymes. Usually, the entire gel was stained for one enzyme after the other. Prior to each new staining procedure, the gel was rinsed in water for 10-20 seconds. In some cases it was appropriate to slice the gel perpendicularly to the pH gradient in order to enable two stainings simultaneously. Gels were depicted on a light table immediately after each staining procedure.

A series of experimental trials were performed in order to determine the most favorable tissue type, gel type, running time and stain recipe. Trials typically started with starch gels, followed by isoelectric focusing on wide pH ranges. 16 enzyme systems were stained for, and the applied tissues, gel types, running conditions and stain recipes are presented in table 1. Both staining procedures and recipes were modified from Shaw and Siciliano (1976), Shaw and Prassad (1970) and Allendorf *et al.* (1977).

The stain buffer used was a 0,2M Tris solution, adjusted to pH 8,0 with HCl.

## INTRODUCTION

The harp seal has, together with the hooded seal, been the major target species of Norwegian sealing since its beginning in the 1830's. Main sealing areas for the harp seal are Newfoundland, in the Jan Mayen area and in the Eastern Barents Sea. The harvesting of harp seals traditionally began in the reproduction season and continued to the end of moulting in the month of May. The catch of pups is presently forbidden in the Newfoundland and Jan Mayen areas, where catches therefore initiate later in the spring. Aggregation in breeding areas starts in late February or early March in the White Sea and at Newfoundland, and about 3 weeks later at Jan Mayen.

Although more is known about the harp seal than virtually all other sea mammals, many aspects of their biology remain obscure. Among the most important problems from a management point of view, is to delineate harp seal population structure.

Presently, genetic characters detected by standard gel electrophoresis (i.e. on starch or agar gels) are those most widely used to identify genetically distinct groups of vertebrates. As enzyme systems in Newfoundland and about Jan Mayen harp seals were investigated by starch gel electrophoresis (s.g.e.) by Lavigne *et al.* (1978), only one of 55 screened loci met the 95% criterion for polymorphism in both areas. Another locus only just met the criterion for Newfoundland samples.

These findings led to the conclusion that "Northwest Atlantic harp seals were ... among the least variable of vertebrate species examined to date." (Lavigne *et al.* 1978). Intraspecific comparisons using biochemical data are highly dependent on the presence of polymorphisms. On one hand, s.g.e. has been estimated to detect about one third of the variation in examined structural loci (Lewontin 1974, as cited in Bonhomme and Selander 1978), a resolution capacity which is sufficient for most taxa. On the other hand, for species with particular low genetic variation, s.g.e. may fail to resolve a sufficient number of polymorphic loci, and a high-resolution method could be more adequate. Such a method is developed for detection of microheterogeneities in diagnostic human genetics. Adaptations of this method to large scale investigations of natural populations is suggested in the present work.

The objective of this paper has been to compare samples from different Northeast Atlantic harp seal populations genetically. A combination of standard and alternative methods were employed in order to provide sufficient data for statistical analysis.

## MATERIAL AND METHODS

In 1987, -89 and -90, fresh samples of liver and skeletal muscle from harp seals were collected in the Eastern Barents Sea and in the Jan Mayen area. Sampling was carried out during the period between pupping and moult seasons in the respective areas. When several samples were collected the same year in the same area, Roman numerals were added to the sample denotation. Samples were removed and frozen 0.5-1.5 hours after the animals had been killed. Material from 1987 was stored at -20°C until use, while the material from 1989 and 1990 was stored at -20°C for one to three months, and subsequently frozen at -70°C until use.

Samples were sonified and eventually centrifuged (only for MDH) prior to analysis either by s.g.e. or by isoelectric focusing in precast polyacrylamide gels (IFPAG).

Starch gels (thickness: 10 mm) were made using 13% w/v potato starch. Two buffer systems were used:

a) Bridge: 1208 g. tri-Na-citrate-dihydrate in 10 l dest. water. Adjusted to pH 7,0 with citric acid.

Table 1. Enzymes stained for, applied tissues, gel types (characterized by pH-ranges), running conditions (duration of prefocusing and total running time) and stain recipes.

M and L denote muscle and liver tissue respectively. Starch gel composition is given in the text. 100 ml stain solutions were employed, and if not stated otherwise, the stain buffer given in the text was used. ADP = adenosine diphosphate; G6PDH = glucose-6-phosphate dehydrogenase; MTT = (dimethylthiazol)-2,5-diphenyltetrazolium bromide; NAD = β-nicotinamide adenine dinucleotide; NADP = β-nicotinamide adenine dinucleotide phosphate; PMS = phenazine methosulphate. (1) As MDH should not be focused, the ideal running time was usually between 70 and 80 minutes, depending on the cooling temperature. (2) Exposed to light during incubation

| Enzyme | Enzyme number (E.C.) | Abbre-viation | Tissue | Gel | Running conditions (in minutes) | Stain solution components |
|---|---|---|---|---|---|---|
| Aspartate aminotransferase (glutamic-oxaloacetic transaminase) | 2.6.1.1 | AAT (GOT) | M,L | 5,5-8,5 | 90,180 | 400 mg α-aspartic acid<br>120 mg α-ketoglutaric acid<br>40 mg pyridoxal-5-phosphate<br>-Incubated for 15 min. before adding<br>200 mg Fast Blue BB |
| Alcohol dehydrogenase | 1.1.1.1 | ADH | L | 3,5-9,5 | 10,105 | 13 mg MTT<br>8 mg NAD<br>12 ml ethanol (96%)<br>3 mg PMS |
| Adenylate kinase | 2.7.4.3 | AK | (M,L) | 3,5-9,5 | 10,105 | 13 mg MTT<br>13 mg NADP<br>25 mg $MgCl_2$<br>25 mg ADP<br>125 mg glucose<br>50 µl hexokinase<br>25 µl G6PDH<br>3 mg PMS<br>-in stain buffer:dest. water=1:4 |
| Alkaline phosphatase | 3.1.3.1 | ALP | L | 5,0-6,5 | 50,120 | 50 mg 1-naphtyl Na phosphate<br>125 mg $MgCl_2$<br>-in dest. water<br>-Incubated for 15 min. before adding<br>50 mg Fast Blue RR |
| Creatine kinase | 2.7.3.2 | CK | (M,L) | 3,5-9,5 | 10,105 | 13 mg MTT<br>13 mg NADP<br>25 mg $MgCl_2$<br>65 mg creatine phosphate<br>30 mg ADP<br>125 mg glucose<br>50 µl hexokinase<br>25 µl G6PDH<br>3 mg PMS |
| Esterase | 3.1.1.- | EST | M | 5,5-8,5 | 40,150 | 75 mg a-naphtyl acetate<br>-in 8 ml acetone<br>200 mg Fast Blue RR<br>-in dest. water |
| Glycerol-3-phosphate dehydrogenase (α-glycerophosphate dehydrogenase) | 1.1.1.8 | G3PDH (α-GPD) | L,M | 3,5-9,5 | 10,105 | 25 mg MTT<br>13 mg NAD<br>625 mg DL-α-glycerophosphate<br>3 mg PMS |

| | | | | | | |
|---|---|---|---|---|---|---|
| Glucose-6-phosphate isomerase | 5.3.1.9 | GPI | (M,L) | 3,5-9,5 | 10,105 | 25 mg MTT<br>16 mg NADP<br>75 mg $MgCl_2$<br>33 mg Na-fructose-6-phosphate<br>65 l G6PDH<br>3 mg PMS |
| L-Iditol dehydrogenase (sorbitol dehydrogenase) | 1.1.1.14 | IDDH (SDH) | L | 3,5-9,5 | 10,105 | 25 mg MTT<br>13 mg NAD<br>375 mg D-sorbitol<br>3 mg PMS |
| Isocitrate dehydrogenase | 1.1.1.42 | IDHP (IDH) | M,L | 3,5-9,5 | 10,105 | 25 mg MTT<br>13 mg NADP<br>40 mg $MgCl_2$<br>250 mg DL-isocitric acid<br>3 mg PMS |
| L-Lactate dehydrogenase | 1.1.1.27 | LDH | M,L | 3,5-9,5 | 10, 145 | 25 mg MTT<br>13 mg NAD<br>1 ml DL-Na-lactate<br>3 mg PMS |
| Malate dehydrogenase | 1.1.1.37 | MDH | M | 3,5-9,5 or starch gel (Histi-dine) | 10,75(1) | 25 mg MTT<br>13 mg NAD<br>300 mg malic acid<br>3 mg PMS |
| Malic enzyme ($NADP^+$) | 1.1.1.40 | ME | M | 3,5-9,5 | 10,105 | 25 mg MTT<br>13 mg NADP<br>25 mg $MgCl_2$<br>250 mg malic acid<br>3 mg PMS |
| Nucleoside phosphorylase | 2.4.2.1 | NP | (M,L) | 3,5-9,5 | 10,105 | 200 mg ionosine<br>30 mg NBT<br>200 mg sodium arsenate<br>3,2 units xantine oxidase<br>10 mg PMS<br>-in stain buffer:dest. water=1:4 |
| Phosphoglucomutase | 5.4.2.2 (formerly 2.7.5.1) | PGM | (M,L) | 3,5-9,5 | 10,105 | 25 mg MTT<br>13 mg NADP<br>260 mg $MgCl_2$<br>75 mg K-gluc-1-phosphate<br>25 l G6PDH<br>3 mg PMS |
| Superoxide dismutase | 1.15.1.1 | SOD | M,L | 3,5-9,5 | 10,105 | 25 mg MTT<br>3 mg PMS<br>(2) |

Heterogeneity measures were carried out by G-test (Sokal and Rohlf, 1981). Enzyme names, -numbers and -abbreviations, as well as gene-, locus- and allele symbols are those recommended by Kendall *et al.* (1990). While the previous designation of structural loci and alleles in the harp seal (Lavigne *et al.*, 1978) is based on the migratory properties of proteins under s.g.e. conditions, designations in the present work are based on their isoelectric points (pI). Consistently demonstrable loci and alleles were scored from high pI to low pI; the locus coding for isozymes with highest pI being designated *-1** (e.g. *MDH-1**), and the allele coding for the allozyme with highest pI being designated **a* (e.g. *MDH-1*a*). On condition that the isozyme with the highest pI has the fastest cathodal migration under s.g.e. conditions, this nomenclature is consistent with the one proposed by Lavigne *et al.*, (1978), and in accordance with the recommendation in Kendall *et al.* (1990): "... that

existing practices (if established) be followed for the taxa under study...". (Due to the difference in methodology, alleles in the present work are designated by letters instead of by measures of relative migration distances employed by Lavigne *et al.*, (1978).) Yet, comparisons between results obtained with the different methods should be made with precaution: Notably in starch and acrylamide gels, migratory properties can be affected not only by the the protein's pI, but also by the size and shape of the protein, or by combinations of ions in the electrophoretic medium with uncharged groups of the protein.

## RESULTS AND DISCUSSION

Among the 16 enzymes analyzed in the present study, AK, CK, GPI, NP and PGM failed to demonstrate consistent resolution or staining. The remaining 11 enzymes were encoded at 25 presumptive loci. If not stated otherwise, the description of the following enzymes refers to activity in the tissue type indicated in table 1.

*AAT-1**, *ADH**, *ALP-1**, *EST-1**, *G3PDH-3**, *IDDH**, *IDHP-1** and *-2**, *LDH-2**, *MDH-2**, *ME-2** and *SOD-1** and *-2** demonstrated no individual variation. As illustrated in figure 2, the number of bands in the respective zymograms varied from one (e.g. AAT-1) to six (SOD-1). IDHP resolution was rather poor, and the exact number of bands was difficult to estimate. IDHP-1 was strongest in muscle, while IDHP-2 was strongest and better resolved in liver. SOD-2 was only observed in muscle. *LDH-2** will be described below.
*G3PDH-1** and *-2**, *MDH-1**, and *ME-1** demonstrated at least one heterozygote each, but were not polymorphic at the 95% level. The main G3PDH activity zone consisted of proteins encoded at the *-2** and *-3** loci (fig. 2). One individual possessed the rare *-2*a* allele, and the banding pattern of this heterozygote suggested a dimeric nature of G3PDH-2. Liver tissue expressed an additional zone of activity (G3PDH-1) which consisted of considerably weaker bands (fig. 2). Further, an occurrence of the *-1*b* allele showed that G3PDH-1 was monomeric, not dimeric like G3PDH-2. Due to these diverging features, G3PDH-1 was thought to be an unrelated locus. The same conclusion was drawn by Lavigne *et al.* (1978) without prior knowledge to the difference in quaternary structure between G3PDH-1 and G3PDH-2.

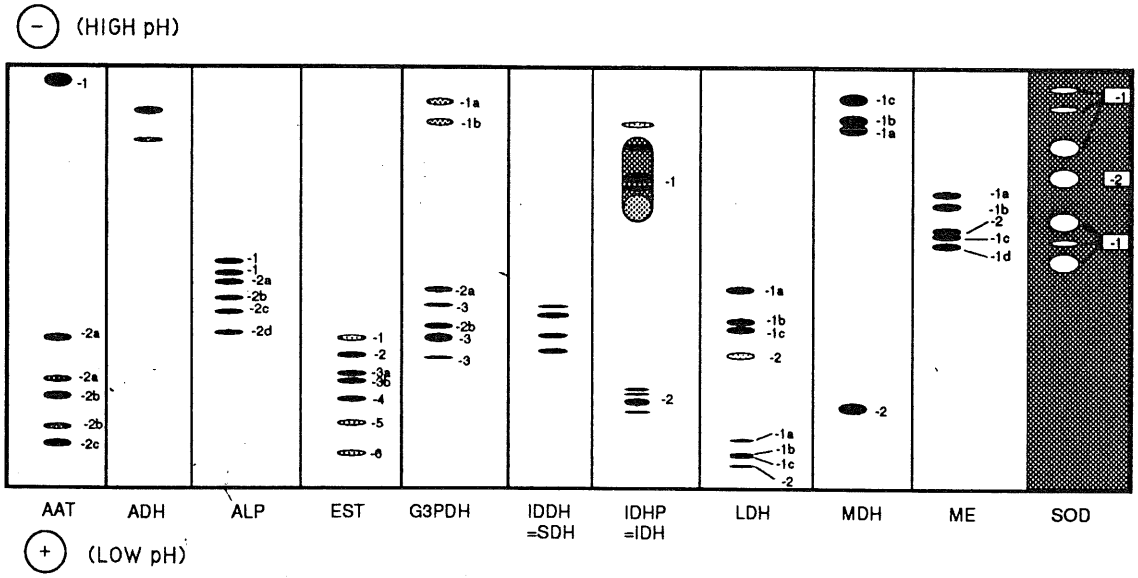



Figure 2. Schematic illustration of zymograms for each of the enzymes that demonstrated consistent resolution and staining. Also presumed satellite bands (artifacts) are illustrated.

The banding patterns observed for *MDH-1** (fig. 2) suggest the presence of a dimeric system with one common (**b*) and two rare (**a* and **c*) alleles. The alleles are probably the same as those detected by Lavigne *et al.* (1978), but the 95% criterion for polymorphism was not satisfied for the combined material. In addition to MDH activity, the stained products of *LDH** were occasionally observed after MDH stainings.
Four alleles were scored for *ME-1** (fig. 2), but the **a*, **c* and **d* alleles constituted a total of less than 5%. The tetrameric nature of this protein was disclosed by **b*d* heterozygotes.

*AAT-2**, *ALP-2**, *EST-3** and *LDH-2** were all polymorphic at the 95% criterion:
*AAT-2**. Liver and muscle tissue expressed the same bands, and the same amount of activity. The banding patterns observed for AAT-2 heterozygotes suggest that this enzyme is dimeric. Yet, zymograms on IFPAG are somewhat complicated by satellite bands (fig. 2). Satellite bands are not observed on starch gels. As in the work by Lavigne *et al.* (1978), one rare allele (**c*) was observed in addition to the common **a* and **b* alleles. G-statistic showed that the sample collected at Jan Mayen in 1989 (J.M.-89) contributed with 41% of the total heterogeneity in the seven analyzed samples, but the result is not significant at the 95% level (tab. 2).

Table 2. G-values based on observed (O) and expected (E) distributions of AAT-2 phenotypes. The rare *c allele was pooled with the *b allele. E.B.S.: Eastern Barents Sea; J.M.: Jan Mayen area.

| SAMPLE | AAT-2 PHENOTYPES | | | | | | | AAT-2 GENE FREQUENCIES | | | G-STAT. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | aa | | bb | | ab | | | | | | | |
| | O | E | O | E | O | E | Σ | a | b | Σ | Gi | d.f. |
| E.B.S.-87 | | | | | | | | | | | | |
| E.B.S.-89,I | 51 | 46,2 | 6 | 6,1 | 29 | 33,6 | 86 | 0,76 | 0,24 | 1,00 | 0,69 | 2 |
| E.B.S.-89,II | 22 | 22,6 | 4 | 3,0 | 16 | 16,4 | 42 | 0,71 | 0,29 | 1,00 | 0,14 | 2 |
| E.B.S.-90,I | 23 | 23,7 | 3 | 3,1 | 18 | 17,2 | 44 | 0,73 | 0,27 | 1,00 | 0,41 | 2 |
| E.B.S.-90,II | 26 | 26,9 | 5 | 3,6 | 19 | 19,6 | 50 | 0,71 | 0,29 | 1,00 | 0,26 | 2 |
| J.M.-87 | | | | | | | | | | | | |
| J.M.-89 | 23 | 18,8 | 3 | 2,5 | 9 | 13,7 | 35 | 0,79 | 0,21 | 1,00 | 1,95 | 2 |
| J.M.-90,I | 26 | 25,8 | 5 | 3,4 | 17 | 18,8 | 48 | 0,72 | 0,28 | 1,00 | 0,25 | 2 |
| J.M.-90,II | 26 | 28,5 | 4 | 3,8 | 23 | 20,7 | 53 | 0,71 | 0,29 | 1,00 | 1,03 | 2 |
| J.M.-90,III | | | | | | | | | | | | |
| Σ | 197 | 192,5 | 30 | 25,5 | 131 | 140,0 | 358 | 0,73 | 0,27 | 1,00 | Σ(Gi): 4,74 | 14 |

*ALP-2**. Zymograms demonstrate a dimeric enzyme and the presence of two common (**a* and **b*), and two rare alleles (**c* and **d*) (fig. 2). The isoelectric points of the respective isozymes were very close, and the polymorphism in *ALP-2** could not be detected with s.g.e.. Alternative alleles for *ALP-2** were not observed by Lavigne *et al.* (1978). Phenotype- and allele distributions are presented in table 3 along with G-values. No significant heterogeneity was observed.

Table 3. G-values based on observed (O) and expected (E) distributions of ALP-2 phenotypes. The rare *c and *d alleles were pooled with the *b allele. E.B.S.: Eastern Barents Sea; J.M.: Jan Mayen area.

| SAMPLE | ALP-2 PHENOTYPES | | | | | | | ALP-2 GENE FREQUENCIES | | | G-STAT. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | aa | | bb | | ab | | | | | | | |
| | O | E | O | E | O | E | Σ | a | b | Σ | Gi | d.f. |
| E.B.S.-87 | 20 | 23,5 | 9 | 11,1 | 38 | 32,4 | 67 | 0,58 | 0,42 | 1,00 | 2,26 | 2 |
| E.B.S.-89,I | 24 | 20,7 | 13 | 9,8 | 22 | 28,5 | 59 | 0,59 | 0,41 | 1,00 | 2,68 | 2 |
| E.B.S.-89,II | 14 | 12,3 | 5 | 5,8 | 16 | 16,9 | 35 | 0,63 | 0,37 | 1,00 | 0,38 | 2 |
| E.B.S.-90,I | | | | | | | | | | | | |
| E.B.S.-90,II | | | | | | | | | | | | |
| J.M.-87 | 8 | 6,0 | 3 | 2,8 | 6 | 8,2 | 17 | 0,65 | 0,35 | 1,00 | 1,19 | 2 |
| J.M.-89 | 10 | 11,6 | 8 | 5,5 | 15 | 15,9 | 33 | 0,53 | 0,47 | 1,00 | 1,20 | 2 |
| J.M.-90,I | | | | | | | | | | | | |
| J.M.-90,II | | | | | | | | | | | | |
| J.M.-90,III | 20 | 21,0 | 8 | 10,0 | 32 | 29,0 | 60 | 0,60 | 0,40 | 1,00 | 0,98 | 2 |
| Σ | 96 | 95,1 | 46 | 45,1 | 129 | 130,9 | 271 | 0,59 | 0,41 | 1,00 | Σ(Gi): 8,68 | 12 |

*EST-3**. EST-3 was expressed in both liver and muscle tissue. The zymogram showed patterns of a monomeric enzyme, and revealed two common alleles, **a* and **b* (fig. 2). An apparent surplus of homozygotes was observed in all samples. The locus was not resolved with s.g.e. by Simonsen *et al.* (1982). 56% of the total heterogeneity in the five analyzed samples was contributed by the J.M.-89 sample mentioned above (tab. 4). The result was not significant.

Table 4. G-values based on observed (O) and expected (E) distributions of EST-3 phenotypes. E.B.S.: Eastern Barents Sea; J.M.: Jan Mayen area.

| SAMPLE | EST-3 PHENOTYPES | | | | | | | EST-3 GENE FREQUENCIES | | | G-STAT. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | aa | | bb | | ab | | | | | | | |
| | O | E | O | E | O | E | Σ | a | b | Σ | Gi | d.f. |
| E.B.S.-87 | | | | | | | | | | | | |
| E.B.S.-89,I | | | | | | | | | | | | |
| E.B.S.-89,II | | | | | | | | | | | | |
| E.B.S.-90,I | 9 | 7,4 | 11 | 9,6 | 14 | 16,9 | 34 | 0,47 | 0,53 | 1,00 | 0,027 | 2 |
| E.B.S.-90,II | 15 | 10,5 | 16 | 13,6 | 17 | 23,9 | 48 | 0,49 | 0,51 | 1,00 | 0,571 | 2 |
| J.M.-87 | | | | | | | | | | | | |
| J.M.-89 | 8 | 7,9 | 18 | 10,2 | 10 | 17,9 | 36 | 0,36 | 0,64 | 1,00 | 4,37 | 2 |
| J.M.-90,I | 14 | 10,1 | 13 | 13,0 | 19 | 22,9 | 46 | 0,51 | 0,49 | 1,00 | 0,611 | 2 |
| J.M.-90,II | 12 | 11,4 | 14 | 14,7 | 26 | 25,9 | 52 | 0,48 | 0,52 | 1,00 | 2,229 | 2 |
| J.M.-90,III | | | | | | | | | | | | |
| Σ | 58 | 47,2 | 72 | 61,2 | 86 | 107,5 | 216 | 0,47 | 0,53 | 1,00 | Σ(Gi): 7,806 | 10 |

*LDH**. In harp seals, somatic LDH is encoded at two autosomal loci; the polymorphic *LDH-1** with the common **b* and **c* alleles, and the monomorphic *LDH-2**. *LDH-2** is to low extent expressed in liver, but supplies somewhat less than half of the LDH subunits in muscle. As the tetrameric LDH may be composed of any combination of LDH-1 and LDH-2 subunits, homozygotes show a five-banded pattern in muscle tissue. Due to very similar isoelectric points in -1b and -1c subunits, the phenotypes were in general difficult to discern. Yet, heterozygotes in muscle are easier to distinguish as they theoretically demonstrate one band for each of the fifteen possible combinations of -1b, -1c and -2 subunits: Under practical conditions, the bands are undistinguishable. The genetic variation has not been detected with s.g.e. (Lavigne *et al.* (1978), Simonsen *et al.* (1982))

Satellite bands are observed on IFPAG, but they focus closer to the anode, and constitute a separate activity zone. This zone was poorly resolved, and the identification of it as a zone of satellite bands was dependent on the appearance of the distinct **a***c* heterozygote. The latter phenotype was observed in one of 325 individuals.

Again, the J.M.-89 sample contributed with the largest fraction of the total heterogeneity (tab. 5), but as in AAT-2 and EST-3, the result was not significant.

Table 5. G-values based on observed (O) and expected (E) distributions of LDH-1 phenotypes. The rare *a allele was pooled with the *b allele. E.B.S.: Eastern Barents Sea; J.M.: Jan Mayen area.

| SAMPLE | LDH-1 PHENOTYPES | | | | | | | LDH-1 GENE FREQUENCIES | | | G-STAT. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | bb | | cc | | bc | | | | | | | |
| | O | E | O | E | O | E | Σ | b | c | Σ | Gi | d.f. |
| E.B.S.-87 | | | | | | | | | | | | |
| E.B.S.-89,I | 12 | 13,3 | 2 | 2,1 | 12 | 10,6 | 26 | 0,69 | 0,31 | 1,00 | 1,02 | 2 |
| E.B.S.-89,II | | | | | | | | | | | | |
| E.B.S.-90,I | 19 | 22,0 | 3 | 3,5 | 21 | 17,5 | 43 | 0,69 | 0,31 | 1,00 | 2,77 | 2 |
| E.B.S.-90,II | 29 | 25,6 | 7 | 4,1 | 14 | 20,4 | 50 | 0,72 | 0,28 | 1,00 | 1,96 | 2 |
| J.M.-87 | | | | | | | | | | | | |
| J.M.-89 | 23 | 18,4 | 1 | 2,9 | 12 | 14,7 | 36 | 0,81 | 0,19 | 1,00 | 3,55 | 2 |
| J.M.-90,I | 25 | 24,6 | 5 | 3,9 | 18 | 19,5 | 48 | 0,71 | 0,29 | 1,00 | 0,03 | 2 |
| J.M.-90,II | 28 | 27,6 | 5 | 4,4 | 21 | 22,0 | 54 | 0,71 | 0,29 | 1,00 | 0,14 | 2 |
| J.M.-90,III | 37 | 34,8 | 10 | 5,5 | 21 | 27,7 | 68 | 0,70 | 0,30 | 1,00 | 1,88 | 2 |
| Σ | 173 | 166,3 | 33 | 26,3 | 119 | 132,3 | 325 | 0,72 | 0,28 | 1,00 | Σ(Gi): 11,35 | 14 |

*EST-2**, *-4**, *-5** and *-6** were obviously polymorphic at the 95% criterion, but were particularly difficult to resolve. Interpretation was complicated by the focusing of different allozymes on a narrow pH range, similar isoelectric points for variant alleles and quantitative differences in stained products between individual samples.

The J.M.-89 sample contributed with the largest fraction of the total heterogeneity in three of the four polymorphic loci described above. The higher G-values for the J.M.-89 sample is mainly caused by diverging gene frequencies, and four scatterplots were carried out in JMP in order to illustrate gene frequencies in two or three loci simultaneously (fig. 3-6). Two-dimensional plots were carried out for five samples (E.B.S.-90,I, E.B.S.-90,II, J.M.-89, J.M.-90,I and J.M.-90,II):

a) *AAT-2*b* frequencies versus *EST-3*b* frequencies (fig. 3).
b) *AAT-2*b* frequencies versus *LDH-1*b* frequencies (fig. 4).
c) *EST-3*b* frequencies versus *LDH-1*b* frequencies (fig. 5).

A three-dimensional plot, combining a), b) and c), was carried out for the same samples (fig. 6).
It should be noted that the location of the axis do not indicate numerical coordinates.

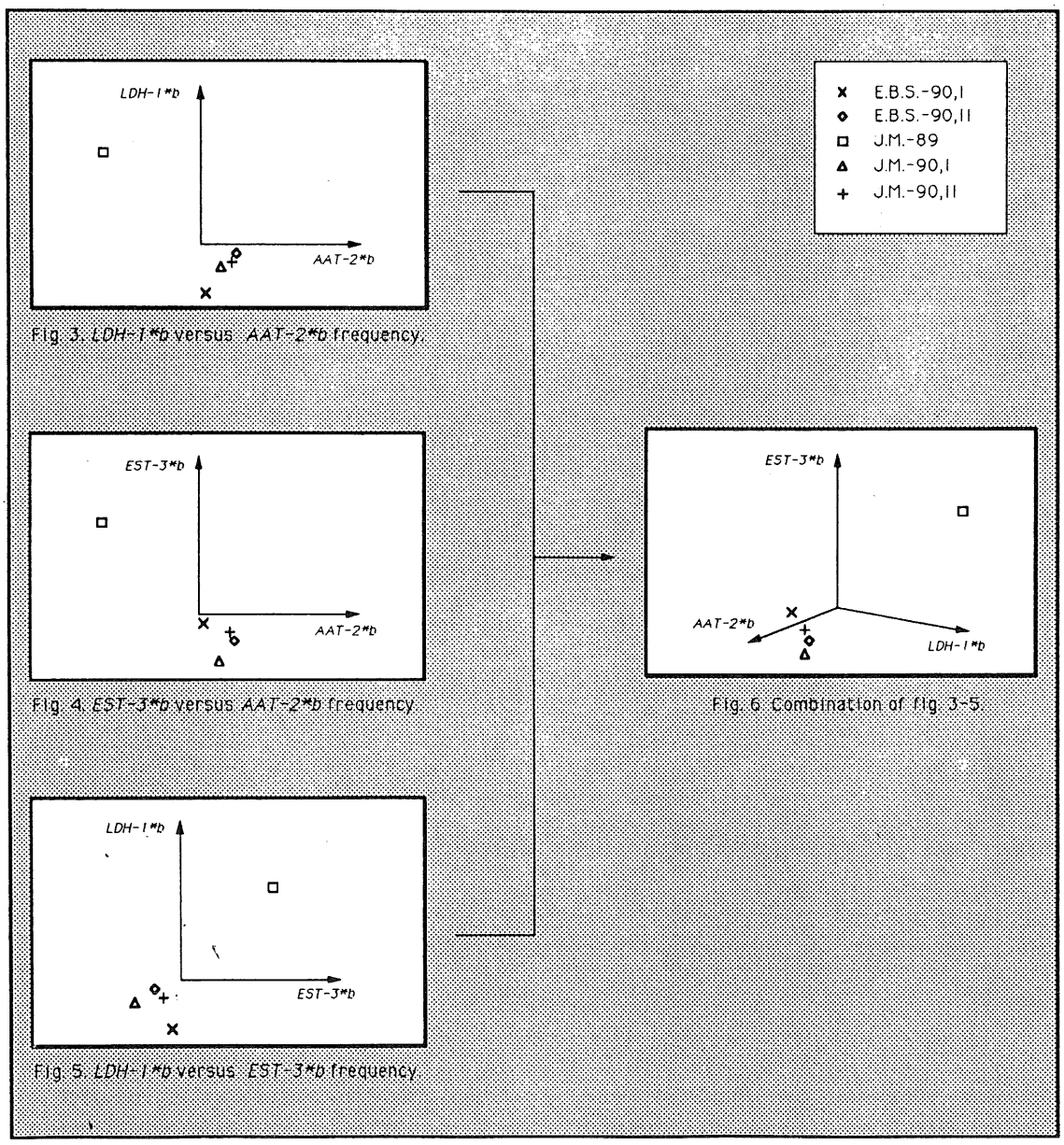



Fig. 3. *LDH-1*b* versus *AAT-2*b* frequency.

Fig. 4. *EST-3*b* versus *AAT-2*b* frequency.

Fig. 5. *LDH-1*b* versus *EST-3*b* frequency.

Fig. 6. Combination of fig. 3-5.

Fig. 3-6 illustrate that in the five samples which have been analyzed for both AAT, EST and LDH, the J.M.-89 sample is an out-layer. A close examination of sample- and sampling characteristics will be made as far as such information is available. Comparisons with polymorphic loci in Newfoundland harp seals will hopefully be carried out, but depends upon the availability of such material.

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