1982

Electrophoretic Variation in Large Mammals. III. The Ringed Seal, Pusa-Hispida, the Harp Seal, Pagophilus-Groenlandicus, and the Hooded Seal, Cystophora-Cristata

V. Simonsen

Fred W. Allendorf
University of Montana - Missoula, Fred.Allendorf@umontana.edu

W. F. Eanes

F. O. Kapel

Hereditas 97: 87–90 (1982)

Electrophoretic variation in large mammals

III. The ringed seal, *Pusa hispida*, the harp seal, *Pagophilus groenlandicus*, and the hooded seal, *Cystophora cristata*.

V. SIMONSEN[1], F. W. ALLENDORF[2], W. F. EANES[3] and F. O. KAPEL[4]

[1] *Institute of Ecology and Genetics, University of Aarhus, Denmark*
[2] *Department of Zoology, University of Montana, USA*
[3] *Department of Ecology and Evolution, State University of New York, Stony Brook, USA*
[4] *Greenland Fisheries Investigations, Charlottenlund, Denmark*

SIMONSEN, V., ALLENDORF, F. W., EANES, W. F. and KAPEL F. O. 1982. Electrophoretic variation in large mammals. III. The ringed seal, *Pusa hispida*, the harp seal, *Pagophilus groenlandicus*, and the hooded seal. *Cystophora cristata*. — *Hereditas 97*: 87–90. Lund, Sweden. ISSN. 0018-0661. Received January 4, 1982

Twenty-one enzyme loci have been resolved by starch gel electrophoresis from liver and muscle tissue of 82 ringed seals, 6 harp seals, and 10 hooded seals. Four loci were polymorphic in the ringed seal, but only one in the harp seal and the hooded seal. The genetic variability is, thus, low as in many large mammals.

Vibeke Simonsen, Institute of Ecology and Genetics, University of Aarhus, Ny Munkegade, DK-8000 Århus C, Denmark

The intention of the present study is to examine seal species in order to test the proposal that low genetic variation as revealed by starch gel electrophoresis is a general phenomenon among carnivores; see SIMONSEN (1982); BONNELL and SELANDER (1974); MCDERMID et al. (1972); and ALLENDORF et al. (1979). This study includes three North Atlantic seal species. The ringed seal (*Pusa hispida*) is a true Arctic species, which breeds in snow-covered lairs on solid ice and occurs single or in small groups all through the year. The harp seal (*Pagophilus groenlandicus*) and the hooded seal (*Cystophora cristata*), on the other hand, whelp in large concentrations on the open drift ice near Newfoundland, Jan Mayen, or in the White Sea, and conduct large annual migrations to and from feeding areas in the Arctic. All three species are rather long-living and, as a rule, give birth to a pup each year from the time when they attain maturity, at the age of 3 to 7 years.

Comparative chromosome studies in Pinnipedia have been carried out by FAY et al. (1967); ARNASON (1974); and by ÁRNASON (1977). The karyotypes of the pinnipeds are characterized by a pronounced uniformity. The diploid chromosomal number for the ringed and the harp seal is 2n=32, whereas the hooded seal has 2n=34. The 2n=32 phocid karyotype has evolved from the 2n=34 karyotype by a two-pair fusion, which has been demonstrated by ARNASON (1974). All the karyological analyses support the theory of monophyletic origin of the Pinnipedia. Immunological measurements of structural differences among various carnivore albumins also support the hypothesis; see SARICH (1969).

Studies on hemoglobins among pinnipeds by use of electrophoresis show that all the pinnipeds analysed have a major band in common, but the second major band has a migration rate shared by walrus, fur seal, and sea lion, and another for grey, harbor, and ribbon seal; see LINCOLN et al. (1973). This similarity in the zymograms of hemoglobin from carnivores led SEAL (1969) to propose that all species tested in six carnivore families (*Canidae*, *Procyonidae*, *Ursidae*, *Mustelidae*, *Otariidae*, and *Phocidae*) have a major hemoglobin component of identical primary structure, but this statement has been heavily attacked by SARICH (1972). SARICH (1972) has tested the hemoglobin of the dog (*Canis familiaris*) with the microcomplement fixation procedure and has observed that each non-Canis hemoglobin tested reacted less well than that of the dog, and each cross-reaction value was unique. Observing the same electrophoretic form of a given protein from two different species does not mean that the amino acid se-

quence of the protein is identical in the two species.

Previous electrophoretic studies on North Atlantic seals by NÆVDAL (1966a, b) reveal nearly no variation in hemoglobin for the harp, the bearded, the ringed, and the hooded seal, but transferrin is polymorphic in the harp seal. For the genotypic distributions in transferrin from different sampling areas, no significant differences are observed; see NÆVDAL (1966a, 1969).

More comprehensive electrophoretic studies have been done on the Macquarie Island elephant seal by MCDERMID et al. (1972) and on the northern elephant seal by BONNEL and SELANDER (1974). Both studies reveal very little genetic variation in these species. BONNELL and SELANDER (1974) have explained the low genetic variation of the northern elephant seal as the result of a population bottleneck in the nineteenth century. MCDERMID et al. (1972) have offered no genetic interpretation of the observed low genetic variation.

Materials and methods

The tissues used for this study were from seals collected in Greenland 1974, 1975, and 1976 by Dr. Christian Vibe, Zoological Museum, Copenhagen and by F. O. Kapel and J. Christiansen for Greenland Fisheries Investigations, and the Ringed Seal Project sponsored by the Danish Natural Science Research Council. Liver and muscle samples were removed shortly after the animals had been killed, and were stored at −18°C or −70°C until use. A total of 82 specimens of the ringed seal, 6 of the harp seal, and 10 of the hooded seal were collected.

The samples were analysed by starch gel electrophoresis; buffer systems and staining procedures were the same as described by SIMONSEN (1982).

Results

For the three species of seals the results of the enzyme analysis are reported below.

Acid phosphatase (ACP). — Liver tissue revealed the greatest amount of activity. The enzyme consisted of one heavy band and some minor bands, which were not possible to score. There was no variation in migration of the heavy bands within or between the three species.

Adenosine deaminase (ADA). — Liver tissue exhibited the greatest amount of activity. All three seal species had a single zone of activity, which migrated an equal distance for all the species.

Adenylate kinase (AK). — Activity was found in both liver and muscle tissues. Muscle tissue expressed one heavily stained band and two minor bands. No variation among the species was observed.

Catalase (CAT). — Activity was present in both tissues. Only one band was seen and no variation existed among the species.

Diaphorase (DIA). — Liver samples revealed the greatest activity. Two bands were found and no variation within or among the species was observed.

Esterase (EST). — Two zones of activity were found in liver tissue, and the same two zones were found in muscle tissue. Staining with 4-MUB-acetate revealed no additional zones of activity. No variation among the species was observed.

Glucose-6-phosphate dehydrogenase (G6PD). — Muscle tissue showed a single heavy activity band, whereas liver tissue expressed a rather diffuse zone of activity. There were no differences in the migration for G6PD among the muscle samples from the three species.

Glutamic-oxaloacetic transaminase (GOT). — Both tissues possessed two bands of activity. No variation in the species was seen, but the anodic migrating band from the hooded seal migrated faster towards the anode than the anodic band of the other two species, which comigrated.

Glutamic-pyruvic transaminase (GPT). — Both liver and muscle tissue expressed enzyme activity. One main zone and two weaker zones of activity were observed. No variation among the species or within the species was seen.

Isocitrate dehydrogenase (ICD). — A single band was found in liver tissue and two bands in muscle tissue. No variation among the species was obtained.

Lactate dehydrogenase (LDH). — As expected, both liver and muscle tissue expressed five bands. The band that migrates fastest towards the anode was rather weak in several of the samples. No variation among the species was observed.

Malate dehydrogenase (MDH). — Both liver and muscle tissue exhibited two zones of activity, each with a heavily stained band and one to two additional, more weakly stained bands. The two zones are interpreted as products of two loci, separately termed *MdhI* and *MdhII*. A single ringed seal expressed variation in *MdhI* (a slower migrating phenotype), and for the *MdhII* locus a single ringed seal showed a faster migrating phenotype. A third ringed seal expressed a mixture of the common and rare phenotype of the *MdhII* locus. No further variation was found.

The variation seen in the *MdhI* locus in the ringed seal is unusual. It can be explained by assuming one locus with two alleles, one dominant (the common phenotype), and one recessive (the rare phenotype), because no heterozygotes were observed, and because it is unlikely to get a sample of 82 specimens consisting of 81 common homozygotes and 1 rare homozygote, if the genetic interpretation is one locus with two codominant alleles. Anyhow, this model is not the only one which can explain the observation, but with the information available is a possibility.

Peptidase (PEP). All hooded seals possessed a single activity band both in the liver and in the muscle tissue. In the ringed seal, fourteen specimens had two bands and 68 seals had a single band identical in mobility to the fast band seen in the hooded seal. The genetic interpretation is that the double-banded phenotype is heterozygous and the single-banded phenotype is homozygous for PEP. Among the harp seals, one heterozygote was found, with the remainder possessing a zymogram identical to that seen in the hooded seal.

6-phosphogluconate dehydrogenase (PGD). In the ringed and the hooded seals, both liver and muscle tissue expressed variation. Two of the hooded seals were heterozygous, and the remainder homozygous. One ringed seal was a heterozygote. Regardless of species, all homozygotes migrated with the same speed. The heterozygotes among the hooded seals expressed an additional band migrating towards the anode; however, the heterozygous ringed seal showed a band migrating slower towards the anode than the band seen in homozygotes.

Phosphoglycerate kinase (PGK). Only muscle tissue revealed activity, and all individuals expressed the same zymogram, which consisted of two identically migrating bands in all species.

Discussion

The results are compiled in Table 1, which shows the enzymes studied, the genetic interpretation, and the frequency of the common allele. Four loci in the ringed seal are polymorhpic, and one in each of the other two species.

The proportion of polymorphic loci in the three species of seals is extremely low; at the 0.99 criterion level for the most frequent allele, the proportion of polymorphic loci is 0.095 for the ringed seal, and only 0.048 for the harp and hooded seals. The individual heterozygosity estimates for the species are 0.009 for the ringed and hooded seal, and 0.007 for the harp seal. As for the great apes (see BRUCE and AYALA 1979) and several carnivores, as mentioned in the introduction, the proportion of polymorphic loci and individual heterozygosity is rather low among the three species of seals.

It seems apparent now that the level of genetic variation in the large mammals (see REUTERWALL and RYMAN 1979), is substantially reduced in comparison to small mammals such as the house mouse (see SELANDER et al. 1969). The lowered genetic variation seen in the large mammals is often explained as the consequence of population bottle-necks, as proposed by BONNELL and SELANDER (1974). However, it seems unlikely that each of the large mammals studied, including man, would have passed through a recent bottle-neck. Other reasons for low genetic variation among the large mammals must now be considered, and several of these are discussed in ALLENDORF et al. (1979).

Calculating the genetic distance according to NEI (1972) for the three species we obtain the following results D (ringed versus harp seal) = 0.000, D (ringed versus hooded seal) = 0.050, and D (harp versus hooded seal) = 0.050. These results are in accordance with the expectations from studies of karyotypes and of classification based on morphology, as reported by ARNASON (1974) and MAXWELL (1967), respectively.

The conclusion of this study is that the genetic variation among and within the three species of seals is extremely low, as in several carnivore species.

Acknowledgments. — This work was sponsored by the Committee for Biological Research in Greenland supported by The Danish Natural Science Research Council. We are grateful to the technical staff at the Institute of Ecology and Genetics, for excellent assistance.

Table 1. Enzymes studied in three species of seals, designation of the loci, number of alleles observed, and frequency of the most common allele. Number of individuals of the species is in parentheses

Enzyme	Designation of locus	Number of alleles (and designation)			Frequency of the common allele, (and designation)		
		Ringed (82)	Harp (6)	Hooded (10)	Ringed (82)	Harp (6)	Hooded (10)
Acid phosphatase	*Acp*	1	1	1	1.000	1.000	1.000
Adenosine deaminase	*Ada*	1	1	1	1.000	1.000	1.000
Adenylate kinase	*Ak*	1	1	1	1.000	1.000	1.000
Catalase	*Cat*	1	1	1	1.000	1.000	1.000
Diaphorase	*Dia I*	1	1	1	1.000	1.000	1.000
	Dia II	1	1	1	1.000	1.000	1.000
Esterase	*Est I*	1	1	1	1.000	1.000	1.000
	Est II	1	1	1	1.000	1.000	1.000
Glucose-6-phosphate dehydrogenase	*G6pd*	1	1	1	1.000	1.000	1.000
Glutamic-oxaloacetic transaminase	*Got I*	1	1	1	1.000	1.000	1.000
	Got II	1	1	1	1.000	1.000	1.000
Glutamic-pyruvic transaminase	*Gpt*	1	1	1	1.000	1.000	1.000
Isocitrate dehydrogenase	*Icd I*	1	1	1	1.000	1.000	1.000
	Icd II	1	1	1	1.000	1.000	1.000
Lactate dehydrogenase	*Ldh I*	1	1	1	1.000	1.000	1.000
	Ldh II	1	1	1	1.000	1.000	1.000
Malate dehydrogenase	*Mdh I*	2 (a, b)	1 (a)	1 (a)	0.998 (a)	1.000	1.000
	Mdh II	2 (a, b)	1 (b)	1 (b)	0.982 (b)	1.000	1.000
Peptidase	*Pep*	2 (a, b)	2 (a, b)	1 (a)	0.915 (a)	0.917 (a)	1.000
6-Phosphogluconate dehydrogenase	*Pgd*	2 (b, c)	1 (b)	2 (a, b)	0.994 (b)	1.000 (b)	0.900 (b)
Phosphoglycerate kinase	*Pgk*	1	1	1	1.000	1.000	1.000

Literature cited

ALLENDORF, F. W., CHRISTIANSEN, F. B., DOBSON T., EANES, W. F. and FRYDENBERG, O. 1979. Electrophoretic variation in large mammals. 1. The polar bear, *Thalarctos maritimus*. — *Hereditas 91*: 19–22

ÁRNASON, Ú. 1974. Comparative chromosome studies in Pinnipedia. — *Hereditas 76*: 179–226

ÁRNASON, Ú. 1977. The relationship between the four principal pinniped karyotypes. — *Hereditas 87*: 227–242

BONNELL, M. L. and SELANDER, R. K. 1974. Elephant seals: Genetic variation and near extinction. — *Science 814*: 908–909

BRUCE, E. J. and AYALA, F. J. 1979. Phylogenetic relationships between man and the apes: Electrophoretic evidence. — *Evolution 33*: 1040–1056

FAY, F. H., RAUSCH, V. R. and FELTZ, E. T. 1967. Cytogenetic comparison of some pinnipeds (Mammalia: Eutheria). — *Can. J. Zool. 45*: 773–778

LINCOLN, D. R., EDMUNDS, D. T., GRIBBLE, T. J. and SCHWARTZ, H. C. 1973. Studies on the hemoglobins of pinnipeds. — *Blood 41*: 163–170

MAXWELL, G. 1967. Seals of the World. — *Constable, London*

MCDERMID, E. M., ANANTHAKRISHAN, R. and AGAR, N. S. 1972. Electrophoretic investigation of plasma and red cell proteins and enzymes of Macquarie Island elephant seals. — *Anim. Blood Groups Biochem. Genet. 3*: 85–94

NEI, M. 1972. Genetic distance between populations. — *Am. Natur. 106*: 283–292

NÆVDAL, G. 1966a. Protein polymorphism used for identification of harp seal populations. — *Årbok Univ. Bergen 1965 Mat. — Nat. Ser. 9*: 1–20

NÆVDAL, G. 1966b. Hemoglobins and serum proteins in four North Atlantic seals, studied by electrophoresis. — *Fisk Dir. Skr. Ser. Hav Unders. 14*: 37–50

NÆVDAL, G. 1969 Blood protein polymorphism in harp seals off eastern Canada. — *J. Fish. Res. Bd. Canada 26*: 1397–1399

REUTERWALL, C. and RYMAN, N. 1979. Genetic variability and differentiation in moose in Fennoscandia (*Alces alces alces* L.) and Canada (*A. a. americana* Clinton). — *Hereditas 91*: 312–323

SARICH, V. M. 1969. Pinniped phylogeny. — *Syst. Zool. 18*: 416–422

SARICH, V. M. 1972. On the nonidentity of several carnivore hemoglobins. — *Biochem. Genet. 7*: 253–258

SEAL, U. S. 1969. Carnivora systematics: A study of hemoglobins. — *Comp. Biochem. Physiol. 31*: 799–811

SELANDER, R. K., HUNT, W. G. and YANG, S. Y. 1969. Protein polymophism and genic heterozygosity in two European subspecies of the house mouse. — *Evolution 23*: 379–390

SIMONSEN, V. 1982. Electrophoretic variation in large mammals. II. The red fox, *Vulpes vulpes*, the stout, *Mustela erminea*, the weasel, *Mustela nivalis*, the pole cat, *Mustela putorius*, the pine marten, *Martes martes*, beech marten, *Martes fiona*, and the badger, *Meles meles*. — *Hereditas 96*: 299–305