Low protein variability and genetic similarity between populations of the polar bear (*Ursus maritimus*)

THOR LARSEN, HÅKAN TEGELSTRÖM, R. KUMAR JUNEJA AND MITCHELL K. TAYLOR

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Blood samples from a total of 460 polar bears (*Ursus maritimus*) from various Arctic regions, but excluding the USSR, were collected during the period 1967–1981 to study electrophoretic variation in different proteins. Two hundred and one samples from Alaska, 48 from the Canadian Arctic, 89 from Svalbard, and 21 from Northeast Greenland were collected during the period 1967–1973 and were analysed by vertical polyacrylamide gel electrophoresis to study transferrin and hemoglobin polymorphism. Thirty-one samples collected in 1974 were analysed by starch gel electrophoresis for 14 enzyme systems in serum and red blood cells. Seventy samples collected from Alaska, the Barents Sea, and Canada in 1980–81 were studied by starch gel electrophoresis, and further analysed for protein variation by thin-layer isoelectric focusing, horizontal polyacrylamide gel electrophoresis, and two-dimensional electrophoresis. In all, about 75 loci were analysed for variation. The degree of protein and enzyme variation in the polar bear was observed to be relatively low. Starch gel electrophoresis revealed variation of an unidentified serum protein. The distribution of this protein indicates a closer connection between bears in Alaska and Canada compared to those in Greenland and Svalbard, but the differences were not significant. As in many large mammals, the information from protein variation in polar bears has limited use for management purposes. We could not find any simple system usable for identification of discrete populations. On the basis of protein variation as sole criterion, the populations investigated could not be separated. Possible explanations for the uniformity of blood proteins can be exchange of bears between geographical areas and/or a high selective pressure in polar bears.

Thor Larsen, Norsk Polarinstitutt, Box 158, N-1330 Oslo Lufthavn, Norway; Håkan Tegelström, Department of Genetics, University of Uppsala, Box 7003, S-75 007 Uppsala, Sweden; R. Kumar Juneja, Department of Animal Breeding and Genetics, The Swedish University of Agricultural Sciences, S-75 007 Uppsala, Sweden; Mitchell K. Taylor, Faculty of Forestry, University of British Columbia, Vancouver, British Columbia, Canada. *May 1983 (revised June 1983).*

Introduction

Although polar bears (*Ursus maritimus*) were effectively protected through the International Agreement on the Protection of Polar Bears in 1973, there is still a substantial harvest in Alaska, Canada and Greenland. There is thus increasing concern that overhunting may take place in some countries, or that industrial activities may harm polar bears in some areas. Proper management and conservation require that the borders of discrete populations are identified and defined, and that the exchange of bears between areas is quantified. Information about the extent of effective dispersal, i.e. geneflow between possible polar bear populations, is quite scarce.

Pedersen (1945) claimed that polar bears belonged to one uniform population that migrated clockwise around the Polar Basin. Polar bear mark/recapture programs and satellite telemetry studies have shown that polar bears belong to several, relatively discrete, populations (Larsen 1971; Lentfer 1974; Stirling *et al.* 1977, 1978, 1980; Stirling & Kiliaan 1980; Uspensky & Belikov 1981; Vibe 1982). Many nations share polar bear populations, and there is often a migration and an exchange of bears across jurisdictions (Parovshchikov 1967; Kolz *et al.* 1978; Larsen *et al.* 1980; Vibe 1982). Manning (1971) and Wilson (1976) found geographical variation in polar bear skulls from various areas, but it is not known whether such variation is genotypic.

Genetic variants of different body proteins may be usable as a powerful tool for assessing affinities between different populations of a species. Studies of many species of large mammals, however, have often shown a low level of protein variability, thereby decreasing the possible use of these methods. An extremely high interpopulation genetic similarity was observed in black bears (*Ursus*

Table 1. Summary of materials and methods used for the study of polar bear proteins.

Locality	Year of collecting	Sample size	Method used	Proteins studied
Alaska	1967–1973	201*	VPAGE	Transferrin and hemoglobin
Canadian arctic	1967–1973	48	VPAGE	Transferrin and hemoglobin
Svalbard	1967–1973	89	VAPGE	Transferrin and hemoglobin
East Greenland	1973	21	SGE	Transferrin and hemoglobin
Alaska	1974	10	SGE	14 enzymes and 3 serum proteins
East Greenland	1974	10	SGE	14 enzymes and 3 serum proteins
Svalbard	1974	11	SGE	14 enzymes and 3 serum proteins
Hudson Bay, Canada	1980	24	IEF, SGE, HPAGE, 2-DE	General proteins
Barents Sea	1980	23	IEF, SGE, HPAGE, 2-DE	General proteins
Alaska	1981	23	IEF, SGE, HPAGE, 2-DE	General proteins

* Killed bears.

americanus) (Manlove *et al.* 1980) and in polar bears from East Greenland (Allendorf *et al.* 1979).

The objective of our work was to study and compare possible variation in polar bear blood proteins and enzymes from several Arctic areas.

Material and Methods

A total of 460 polar bear blood samples were collected and analysed between 1967 and 1981 (Table 1). Samples from killed bears were taken from the heart, a vein or an artery. Heparin was added to blood drawn from the femoral vein of the live captured bears. Samples were separated by centrifugation shortly after collection, frozen, and stored at −20°C. When a centrifuge was not available, samples were kept in a cool place for 24 hours to be separated by sedimentation before transfer to vials and freezing.

Vertical polyacrylamide gel electrophoresis (VPAGE) (Raymond 1962) was performed in a Tris-borate-EDTA buffer 0.1-M with pH 9.1 and a 6% polyacrylamide gel (Cyanogum 41, Fisher Scientific Co.). After a prerun of one hour at 250 volts, plasma or serum was pipetted out in slots in the gel. Before application, a 5% solution of 1% bromophenol blue in buffer saturated with sucrose was added to the sample. Erythrocyte samples were washed three times with physiological saline; water and 1 ml ether were added and the samples centrifuged. The supernatant was drawn off and the cells were frozen. Before application to the gel, sucrose crystals were added to the samples.

The electrophoresis was run at 100–150 volts for ten minutes, then at 250–300 volts for about three hours. Serum or plasma proteins were stained with 9 g Amido black dissolved in 400 ml water, 400 ml methanol, and 100 ml acetic acid for 20 minutes. Destaining was performed for 12 hours in the same solution, omitting the Amido black.

All samples were collected in 1974 and were later investigated with the use of starch gel electrophoresis (SGE). Twelve per cent starch gels were used in a Tris-citrate lithium borate buffer of pH 8.5 or a Tris-borate-EDTA buffer of pH 7.5. The separations were performed at 10 V/cm for three hours. Proteins and enzymes were stained in accordance with the techniques rou-

tinely used (Shaw & Prasad 1970; Harris & Hopkinson 1976). Selected samples of serum were treated according to Coppenhauer & Buettner-Janusch (1970) with neuraminidase to determine whether the observed variation in serum proteins was induced by sialic residues (Chen & Sutton 1967). The serum transferrins were also partly purified by rivanol treatment according to Boettcher *et al.* (1958).

Seventy samples collected in the period 1980–81 (Table 1) were studied by means of isoelectric focusing (IEF) in addition to SGE. Samples were electrofocused in a pH gradient of 4–6.5, an appropriate range for almost all polar bear serum proteins. Horizontal polyacrylamide gel electrophoresis (HPAGE) (12% acrylamide in separation gel; Tris-citrate-borate buffer pH 9.0) was done according to Gahne *et al.* (1977).

The serum samples were analysed by a method of two-dimensional electrophoresis (2-DE) described by Juneja *et al.* (1981). The first dimension separation in agarose gel (pH 8.6) was followed by a second dimension separation in horizontal polyacrylamide gel (pH 9.0).

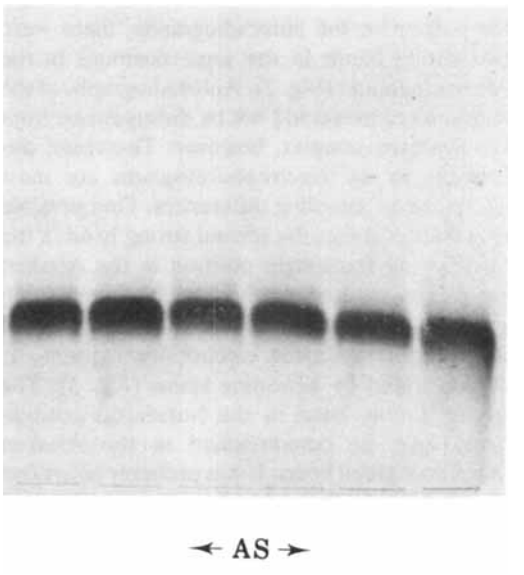

Fig. 1. Vertical polyacrylamide gel electrophoresis of polar bear hemoglobins. Samples from (A) Alaska and (S) Svalbard.

Results

Vertical polyacrylamide gel electrophoresis (VPAGE)

The initial analyses of samples collected before 1974 concentrated on studies of polymorphism in hemoglobins and transferrins by protein separation with VPAGE. The hemoglobin analysis showed an identical pattern, with one single component. No polymorphism could be demonstrated, and there were no visible differences between geographical areas (Fig. 1).

In the serum and plasma electrophoretograms, particular attention was paid to the transferrin component. No transferrin polymorphism could be demonstrated. The serum and plasma separations showed several different general protein patterns, which initially could be suspected to demonstrate transferrin polymorphism. Autoradiography showed that only two of the bands were transferrins, a fast moving, weak component, and a slower, but stronger component. Comparison between the Svalbard samples and the Alaska samples showed differences in the Amido black stained separations. While there was only one strong and one weak transferrin band in the Svalbard samples, corresponding to

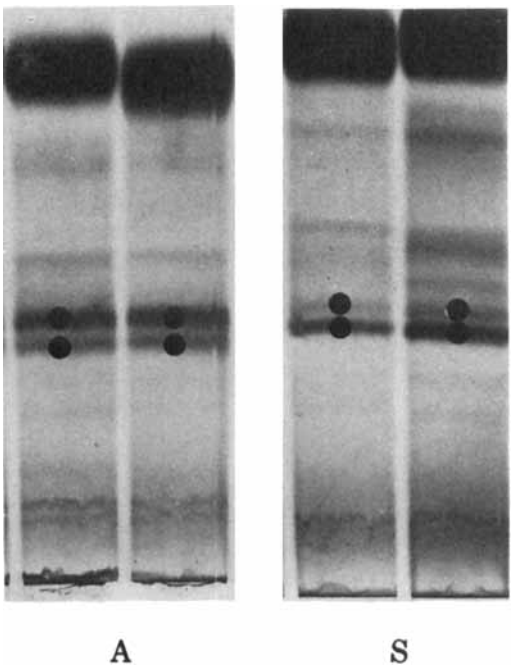

Fig. 2. Vertical polyacrylamide gel electrophoresis of polar bear serum proteins. Samples from (A) Alaska and (S) Svalbard. Transferrins are indicated by dots.

the pattern in the autoradiographs, there were two strong bands in the same positions in the Alaskan samples (Fig. 2). Autoradiographs of the Alaskan samples could not be distinguished from the Svalbard samples, however. The visual differences in the electrophoretograms are most likely due to sampling differences. One possible explanation is that the second strong band in the fast moving transferrin position in the Alaskan samples was caused by a haptoglobin/hemoglobin complex. Such complexes were located close to the transferrins in the electrophoretograms, as demonstrated by benzidine stains (Fig. 3). The strong double band in the transferrin position could only be demonstrated in the Alaskan samples of killed bears. It was probably an artifact caused by hemolysis when bears were shot.

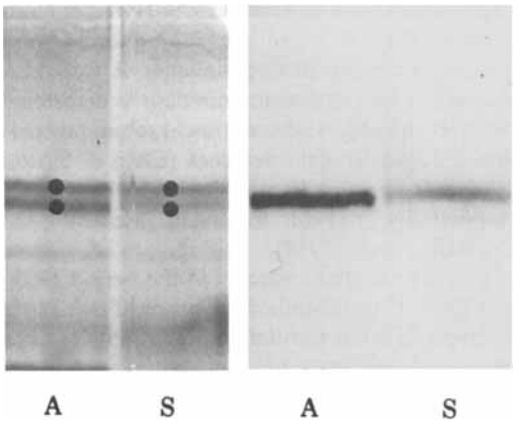

Fig. 3. Vertical polyacrylamide gel electrophoresis of polar bear serum proteins. Samples from (A) Alaska and (S) Svalbard. Autoradiographs (left) show two transferrin bands, indicated by dots. Benzidine stains (right) suggest haptoglobin/hemoglobin complex bindings in the same position as the lowest transferrin band.

Starch gel electrophoresis (SGE)

Red blood cells and serum from 31 samples collected in 1974 were investigated in 1975. Fourteen

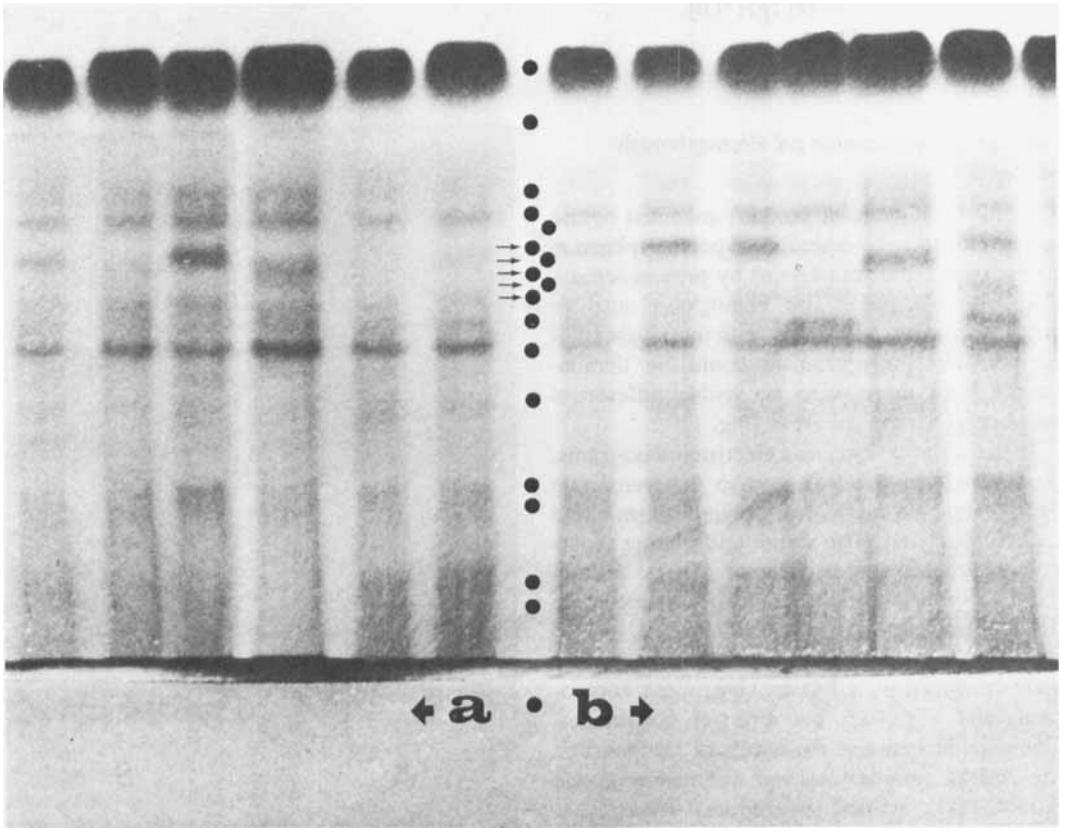

Fig. 4. Starch gel electrophoresis of polar bear serum proteins. Samples from (a) Alaska and (b) Hudson Bay. The variable bands are indicated by arrows.

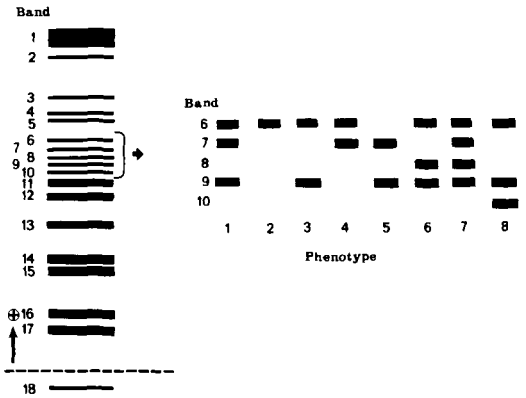

Fig. 5. Interpretation of the variation found in polar bear serum proteins separated by starch gel electrophoresis.

enzymes and three other proteins representing around 30 loci were resolved. The following systems gave good activity and resolution: non-specific esterase, alkaline phosphatase, acid phosphatase, catecol oxidase, catalase, malate dehydrogenase, superoxide dismutase, aldehydeoxidase, isocitrade dehydrogenase, glucose-phosphate isomerase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, NADP-diaphorase, hexokinase, hemoglobin, general proteins, and lipoproteins. Other systems showed only weak activity (leucine aminopeptidase) or exhibited no activity (lactate dehydrogenase, alphaglycerophosphate dehydrogenase, alcohol dehydrogenase, retinol dehydrogenase, betaglucuronidase, and succinate dehydrogenase). The systems showing good resolution were investigated in the 31 bears, and of these 16 systems proved to be monomorphic. In serum, stained for general proteins, there is a system of bands showing variation in all populations investigated. Eighteen protein bands were resolved by SGE of serum (Fig. 4) where bands 11 and 12 represent transferrins. Band 5 was present in some Greenland bears only. Bands 6 to 10 show a phenotypic variation according to Fig. 5, where phenotype 1 is found in Svalbard and Greenland bears. Phenotype 2 was found in all three populations and phenotype 3 was found in Greenland and Alaska bears. Phenotypes 4 and 5 were found in Svalbard bears and phenotypes 6, 7, and 8 were found in Alaska bears. Thus bands 8 and 10 could be found in Alaska bears only. Other bands were found in all populations.

The nature and specificity of the variable serum proteins showing phenotypic variability are unknown and owing to the complexity of the variation observed, a genetic interpretation of the phenotypic variation has not been possible. Bears from Svalbard and Greenland, however, shared the same bands in different combinations in contrast to the bears from Alaska.

General proteins were also investigated in the 70 bears from the period 1980–1981. The same general pattern of variation as in the 1974 samples was found. There was a greater similarity between the Hudson Bay and the Cape Lisburne samples compared to the samples collected in the Barents Sea.

Graphic demonstration starch gel electrophoresis variation is shown in Fig. 5.

Isoelectric focusing (IEF)

Analysis of serum samples yields from 43 to 63 protein bands in pH gradient 4–6.5 (Fig. 6). Individuals from different populations were compared in order to estimate differences between the three populations. Comparisons between separated proteins were performed according to

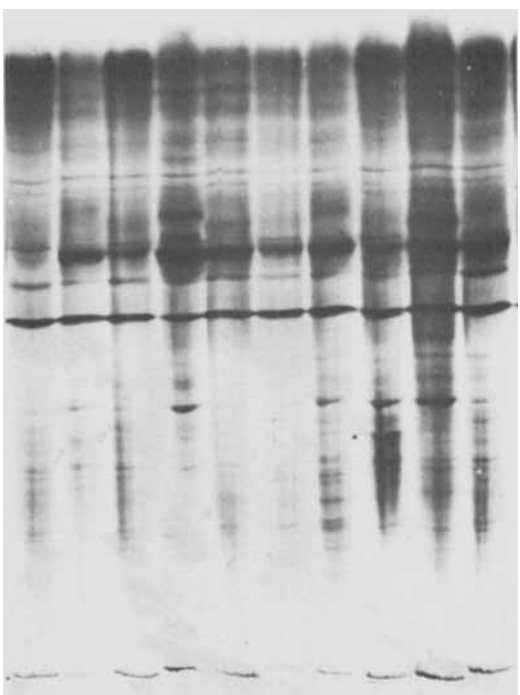

Fig. 6. General protein patterns of polar bear serum separated by isoelectric focusing in a pH gradient of 4–6.5 (lowest pH at top of the gel). Samples from Hudson Bay, Barents Sea and Alaska.

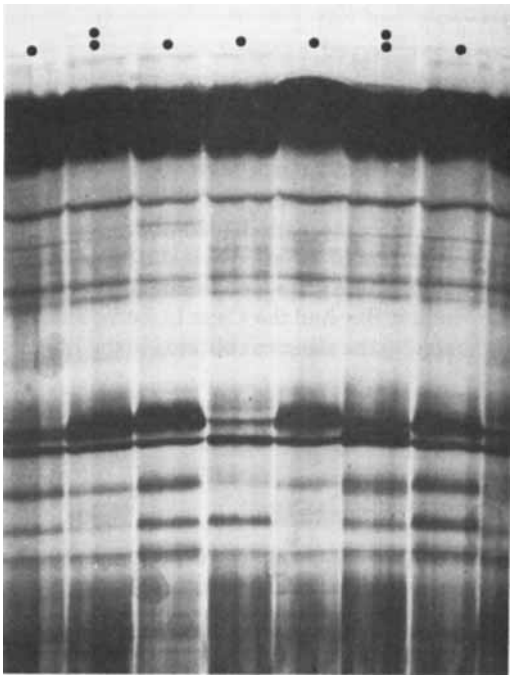

Fig. 7. Horizontal polyacrylamide electrophoresis of polar bear serum proteins. Samples from Alaska. Bands with a presumed genetic variation are indicated. These were also resolved by two-dimensional electrophoresis.

Fig. 8. Two-dimensional electrophoresis of polar bear serum proteins. Samples from Alaska. The bands with a presumed genetic variation found in HPAGE are illustrated with arrows.

Tegelström *et al.* (1982). Identity values between individuals from the same population range from 0.81 to 1.00. Intrapopulational identities estimated for the different populations are: Hudson Bay 0.95 ± 0.03 (n = 8), Cape Lisburne 0.95 ± 0.03 (n = 8), and Barents Sea 0.92 ± 0.06 (n = 8). There is less identity within the Barents Sea material and also a greater statistical variation indicating a more heterogeneous population. Comparisons of individuals from different populations gave the following interpopulation identities: Barents Sea versus Hudson Bay 0.93 ± 0.05 (n = 11), Barents Sea versus Cape Lisburne 0.94 ± 0.05 (n = 12), and Cape Lisburne versus Hudson Bay 0.96 ± 0.04 (n = 13). The identity between populations is very high, and not statistically different from the identity between animals from the same population. There is no statistical identity difference between the three investigated populations. There is, though, an indication of a higher identity between bears from the North American continent compared to the animals sampled in the Barents Sea. With the higher resolution achieved by IEF compared to standard gel electrophoresis, we could not find any protein diagnostic for the populations investigated, although there is variation within populations.

Nonspecific esterases were also investigated by IEF in a pH gradient 3–10, where 11 bands could be resolved. There was no difference between populations.

Horizontal polyacrylamide gel electrophoresis (HPAGE)

Polar bear serum yields from 20 to 27 protein bands (Fig. 7). Variation is present in the different populations, but a genetic interpretation for the observed variations is unlikely. The band which probably represents the α-foeto-protein is divided into two distinct bands in some bears in all populations. The most probable explanation is that the extra band is the result of age and not a genetic difference between individuals. There was variation in a fast moving protein where an extra band was observed in two individuals from the Alaska population. This variation was also observed when the proteins were separated by two-dimensional electrophoresis (Figs. 7 and 8).

Two-dimensional electrophoresis (2-DE)

Patterns of some samples are shown in Fig. 8.

The prealbumin system with probable genetic variation, also found in HPAGE, is indicated. No other variation was revealed by this technique.

All together around 30 enzyme or protein loci were investigated by SGE, and approximately 35 additional proteins and 10 enzymes in serum were resolved by IEF, representing around 45 loci. HPAGE and 2-DE probably revealed some additional proteins not observed by the other two methods. Thus around 75 loci were analyzed all together.

Discussion

Efforts have been made in recent years to evaluate the potential of genetic information in the management of wildlife populations. Levels of genetic variation have primarily been estimated by means of electrophoresis. The method, however, has often proved less useful owing to the usually low level of variability found in large mammals.

Selander & Kaufman (1973) pointed out that vertebrates generally show lower levels of electrophoretically detectable variation than invertebrates. They propose that large and highly mobile animals could show lower levels of variation than smaller and less mobile animals. The level of genetic variation in terms of protein polymorphism seems to be substantially reduced in natural populations of large compared with small mammals (McDermid *et al.* 1972; Bonell & Selander 1974; Allendorf *et al.* 1979; Bruce & Ayala 1979; Ryman *et al.* 1980; Simonsen 1982; Simonsen *et al.* 1982a and b). Several explanations have been proposed (Levins 1968; Selander & Kaufman 1973; Ohta 1974; Valentine 1976).

The low variation found in the present study of 460 polar bears is in agreement with two previous reports on protein and enzyme variation in bears (Manlove *et al.* 1980; Allendorf *et al.* 1979). Manlove *et al.* (1980) investigated 19 proteins in 233 black bears from six localities covering the species range. They found six polymorphic proteins exhibiting two alleles. The low levels of genetic variability in these populations are towards the lower end of the range for mammals. Allendorf *et al.* (1979) investigated 12 enzymes by starch gel electrophoresis of sera and red blood cells in 52 polar bears from eastern Greenland and found no variation.

The degree of polar bear variation in blood proteins and enzymes was found to be low in this study in spite of the use of high resolution techniques. Even proteins usually found to be variable in most species (such as transferrins, GC-vitamin D binding serum protein and non-specific esterases) are monomorphic in this species. Of the 75 loci investigated, SGE revealed an unidentified variable protein, and HPAGE another system. The SGE variation indicates a closer genetic relationship between bears from Alaska and Canada, compared to Greenland and Svalbard. This protein system could not be found in the IEF, HPAGE, or 2-DE, and has not been further investigated. It may be a tool for further identification of polar bear populations.

Extensive mark/recapture programs on polar bears have been made in all Arctic countries since 1966. None of the bears marked in North America have been killed or recaptured in the Eurasian Arctic, or vice versa. Nor have any of the radio-instrumented bears migrated between Europe and North America. The common conclusion of these studies, observations of abundance and distribution, and studies of cranial variation (Manning 1971; Wilson 1976), is that polar bears stay in distinctively different areas for many years. Research suggests the following: Svalbard, Frans Josef Land, Northern Novaja Zemlja and adjacent ice covered seas include the range of one population, which has connections across the Greenland Sea and Northeast Greenland (Larsen *et al.* 1980; Larsen 1981; Parovshchikov 1967). The central East Greenland fjords may have their own small population (Vibe 1981, 1982). In the Canadian Arctic, there may be ten or more populations (Schweinsburg *et al.* 1982; Stirling *et al.* 1977, 1978, 1980; Stirling & Kiliaan 1980; Taylor 1982). Bears in the northeast Canadian Arctic have connection with bears in northwest Greenland (Schweinsburg *et al.* 1982; Stirling *et al.* 1978). In Alaska there may be two different populations (Lentfer 1972, 1974), of which one, in the Chucki Sea, may have connections with bears in the Soviet Wrangel Island and the adjacent Sibirian coast (Kolz *et al.* 1978; Taylor 1982). Bears in the central Soviet Arctic probably belong to a separate population (Uspensky & Belikov 1981). But the discreteness of these populations has been questioned (Prevett & Kolenosky 1982; Stirling *et al.* 1978; Taylor 1982). Observations from many sources show that bears are often encountered in the central Polar Basin, outside their normal range (Lentfer 1970). Such bears may come from, or migrate to, any Arctic region.

Over long periods of time, there may, therefore, be an exchange of bears and hence genetic material sufficient to justify the early statement by Pedersen (1945) that bears belong to one common population, at least in a genetic sense.

The possibility of recent 'bottlenecks' caused by drastic reduction in the number of animals and therefore resulting in lowered levels of genetic variation cannot be rejected, but seems improbable. There is no evidence of any radical reduction in the number of polar bears in any Arctic region in historical time.

It should also be remembered that the polar bear is a relatively young species in the evolutionary sense, and is probably not more than 250,000 years old (Kurtén 1976). Low blood protein and enzyme diversity and lack of differences between populations may be explained by high selective pressure in a species which lives in a difficult environment and a highly specialized niche.

Our conclusion is that the lack of polymorphism in polar bear blood proteins and enzymes reflects the generally low level of protein variability that has been observed in other large mammals. Even if there is little exchange of individuals between polar bear populations, such exchanges may be sufficient to create genetic uniformity over time. This fact, combined with the possible high selective pressure described above, may explain the lack of polymorphism observed in this study.

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