East Greenland and Barents Sea polar bears (Ursus maritimus)

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East Greenland and Barents Sea polar bears (*Ursus maritimus*): adaptive variation between two populations using skull morphometrics as an indicator of environmental and genetic differences

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A morphometric study was conducted on four skull traits of 37 male and 18 female adult East Greenland polar bears (*Ursus maritimus*) collected 1892–1968, and on 54 male and 44 female adult Barents Sea polar bears collected 1950–1969. The aim was to compare differences in size and shape of the bear skulls using a multivariate approach, characterizing the variation between the two populations using morphometric traits as an indicator of environmental and genetic differences. Mixture analysis testing for geographic differentiation within each population revealed three clusters for Barents Sea males and three clusters for Barents Sea females. East Greenland consisted of one female and one male cluster. A principal component analysis (PCA) conducted on the clusters defined by the mixture analysis, showed that East Greenland and Barents Sea polar bear populations overlapped to a large degree, especially with regards to females. Multivariate analyses of variance (MANOVA) showed no significant differences in morphometric means between the two populations, but differences were detected between clusters from each respective geographic locality. To estimate the importance of genetics and environment in the morphometric differences between the bears, a PCA was performed on the covariance matrix derived from the skull measurements. Skull trait size (PC1) explained approx. 80% of the morphometric variation, whereas shape (PC2) defined approx. 15%, indicating some genetic differentiation. Hence, both environmental and genetic factors seem to have contributed to the observed skull differences between the two populations.

Overall, results indicate that many Barents Sea polar bears are morphometrically similar to the East Greenland ones, suggesting an exchange of individuals between the two populations. Furthermore, a subpopulation structure in the Barents Sea population was also indicated from the present analyses, which should be considered with regards to future management decisions.

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The polar bears of East Greenland and those of the Barents Sea area are currently being managed as two separate management units (IUCN PBSG 2010). PAETKAU et al. (1999) studied the genetic structure in 16 of the world's 19 predefined polar bear populations, based on neutral genetic markers; very low level of genetic differentiation was found between the East Greenland and Barents Sea populations, although the allele frequency distributions qualified the two populations as separate management units (MUs) (MORITZ 1994). However, recent observations have questioned the degree of separation between the two populations, suggesting a certain overlap (BORN et al. 2009). Still, differences in space use (BORN et al. 2009) and skull growth rates (BECHSHØFT et al. 2008a), as well as subtle differences in skull, teeth, and bone characters have been found between polar bears from East Greenland and Barents Sea (SONNE et al. 2007a, 2007b; BECHSHØFT et al. 2008b, 2009).

One of the main goals of conservation genetics is the identification of evolutionary significant units (ESUs) – and MUs and the preservation of genetic diversity, which should allow the evolutionary processes of natural selection and adaptation to continue in the future. The identification of separate ESUs was originally recommended based on both ecological and genetic data, although focus has later shifted to being mainly on the assessment

of neutral genetic variation (WAPLES 1991; DEGUIA and SAITOH 2007). However, a broader definition of ESUs including non-neutral markers and adaptive variation would be more appropriate (DEGUIA and SAITOH 2007). Neutral molecular marker loci provide only little insight into adaptive variation, unless a large fraction of the non-neutral markers are tightly linked to the relevant quantitative trait loci. Alternatively, neutral markers can provide useful information in small populations where most of the fitness variation is neutral. However, a lack of molecular divergence among populations at neutral loci is potentially uninformative, as it cannot exclude local adaptations (LYNCH 1996). Neutral genetic markers such as microsatellites have a well-known inheritance but may not be useful in studies of local adaptation, since there is often a limited correlation between neutral and selective variation in natural populations (MERILÄ and CRNOKRAK 2001). Genetic differences, on the other hand, could potentially be measured by proxy instead, as e.g. morphometric variation in physical traits.

Knowledge of local adaptation and adaptive potential of various wildlife populations is becoming increasingly relevant due to impacts on the environment from i.e. climate change and pollution, which could potentially favour the more adaptive species. Divergent natural selection due to spatially varying environments is expected to promote adaptive evolutionary responses (KAWECKI and EBERT 2004). Hence, the evolutionary outcome is dictated by the relative strength of natural selection (ENDLER 1986).

Selective forces influence populations in various parts of their range differently (ANDERSSON 1994). Demonstration that a population is adapted to a given environment is also important (PERTOLDI et al. 2007, 2009). Acquiring direct evidence involves either comparison of fitness among populations in local and foreign environments (KAWECKI and EBERT 2004).

Presently more information is thus wanted on the degree of exchange between the polar bear populations of East Greenland and the Barents Sea. The main aim of the present study was therefore to compare differences in size and shape of East Greenland and Barents Sea polar bear skulls, exploring the degree of morphometric differentiation between the two populations.

MATERIALS AND METHODS

Samples

The sample consisted of 55 (37 male and 18 female) adult polar bear skulls collected in East Greenland between 1892 and 1968 and 98 skulls (54 male, 44 female) collected in Barents Sea between 1950 and 1969. All skulls are located at the Zoological Museum, the Natural History Museum of Denmark, ZMUC (East Greenland bears) and

the Natural History Museum, University of Oslo (Barents Sea bears). The skulls had been aged from counting of the cementum growth layer groups (GLG) of the lower right incisor (I3) after decalcification, thin sectioning (14 Am) and staining (Toluidine Blue), using the method described by HENSEL and SORENSEN (1980) and DIETZ et al. (1991). Male bears were categorized as adults at the age of ≥ 6 years, and female bears at the age of ≥ 5 years (ROSING-ASVID et al. 2002). In a few instances the sex of individual bears was determined using the canonical discriminant functions given in BECHSHØFT et al. (2008b). All animals were born ≤ 1960, as determined from age and date of capture (year of kill).

Measurements

Measurements of four skull traits were used: postorbital height (POH), condylobasal length (CBL), mandible length (ML) and mandible height (MH). The traits were measured using digital callipers (Mitutoyo, Mitutoyo Corporation, Japan) to the nearest 0.04 mm and are defined in detail in BECHSHØFT et al. (2008b). Measurement error for each of the traits can be found in BECHSHØFT et al. (2008c). Some skulls were too damaged for all traits to be measured. Missing data was replaced with the population average (in less than 5% of the traits measured).

Statistical analyses

Polar bears are sexually dimorphic, both in size and shape (DEROCHER et al. 2005; BECHSHØFT et al. 2008a), and hence the sexes were kept separate in all analyses.

Mixture analysis

Mixture analyses were conducted using the four skull traits measurements, keeping the sexes separate, using the software EMMIX (described in MCLACHLAN and PEEL 1998), which fits t-component mixture models to multivariate data, and does not require specifications of any parameters prior to the analysis. The analyses were performed with unrestricted variance-covariance matrices and 10 000 random and 10 000 K-mean starts where 50% of the data were used for every K-mean start.

If a mixture analysis is performed for a range of values of possible clusters (C), the listing for the output file is repeated sequentially for each value fitted for the number of C. Finally, a table is given, summarizing the values of the tests to help decide on the number of clusters (NC) (Table 1). The various criteria currently reported by EMMIX are the log-likelihood (log-lik), the Akaike information criterion (AIC), and the Bayesian information criterion (BIC). The number of clusters is given by the value for which the criterion value is minimized. The criterion value indicates how much disagreement there is between

Table 1. *Mixture analysis output conducted on the Barents Sea polar bear population's four skull traits (see text for details), holding the sexes separated. Sex: Clusters (C). NC: Number of clusters. Log-lik: Log-likelihood. AIC: Akaike information criterion. BIC: Bayesian information criterion.*

Sex	NC	Log-lik	AIC	BIC	*p*
Males	1	−714.1	1456.21	1484.05	–
Males	2	−660.33	1378.66	1436.34	0.01
Males	3	−244.73	577.46	664.97	0.01
Males	4	−231.62	581.24	698.59	0.99
Females	1	−462.18	952.37	977.35	–
Females	2	821.86	−1585.72	−1533.98	0.01
Females	3	856.94	−1625.88	−1547.38	0.04
Females	4	885.96	−1653.92	−1548.65	0.22

the log-lik, AIC, and BIC, which all have different degree of conservativeness. The less disagreement there is between these three indexes, the better it is. The p-value (p) is produced by the optional bootstrap analysis. By sequentially testing e.g. '1 versus 2' then '2 versus 3', and so on, and stopping when the step becomes insignificant ($p > 0.05$), the number of clusters can be assessed. The mixture analysis has the major advantage that it performs an unbiased analysis of the data without any a priori expectations, unlike many other approaches which test the difference of pre-specified groups which may or may not be real, and has been called the only clustering process that is entirely mathematically justifiable (MARRIOTT 1975). The mixture method assumes that the data is composed by a mixture of several clusters and splits the data into these clusters. The method does not use geographical information as an input and the grouping of a significant amount of individuals from the same localities in the same cluster therefore gives strong evidence of a geographic differentiation. The mixture analysis was performed for both East Greenland and Barents Sea bears.

Principal components analysis of the skull measurements of the East Greenland and Barents Sea populations

A principal component analysis (PCA) following MARCUS (1990) was carried out on the covariance matrix derived from the skull measurements. The PCA was executed holding the sexes separate, but pooling the individuals from both areas (East Greenland and Barents Sea).

The results of the PCAs were visualized using the convex hull method.

According to CHASE et al. (2002) the PCA is able to classify phenotypic variation into independent components, which can then be used to dissect genetic networks regulating complex biological systems. If size variation is present in the data and the loadings of principal

component 1 (PC1) are either all positive or negative, PC1 can be said to summarize the within-sample size variation (BOOKSTEIN 1989). Shape, on the other hand, quantifies the variation that cannot be explained by size variation and allometric relationships.

Principal components analysis of the clusters defined by mixture analysis

A PCA was done for the polar bears of East Greenland and Barents Sea subpopulation clusters defined by the mixture analysis (still holding the sexes separate). The results of the PCAs were visualized using the convex hull method.

Multivariate analysis of variance of the skull measurements of the East Greenland and Barents Sea populations

A multivariate analysis of variance (MANOVA) was conducted (holding the geographic locations and the two sexes separated) with an a posteriori Wilk's lambda and a Pillai trace, which were used as indexes of multivariate distances. The analysis was done in order to test the significance of multivariate morphometric differentiations between the polar bear populations of East Greenland and Barents Sea.

Multivariate analysis of variance of the clusters defined by mixture analysis

A MANOVA was used to analyze for significance of morphometric differentiation between the morphometric cluster defined for the population of East Greenland and the clusters defined for the Barents Sea population by the mixture analysis. A Hotelling's test was conducted (using the software PAST, HAMMER et al. 2004) in connection with both the above mentioned MANOVAs.

RESULTS

Mixture analysis

The mixture analysis revealed three significant clusters for Barents Sea males and three significant clusters for Barents Sea females (Table 1). The mixing proportion (number and the percent of individuals assigned to each cluster) in males for each component was: Cluster 1: 12 (23%), Cluster 2: 34 (63%), Cluster 3: 8 (14%) and for females it was: Cluster 1: 22 (51%), Cluster 2: 16 (36%), Cluster 3: 6 (13%). The East Greenland population consisted of one cluster for females (18 individuals, 100%) and one cluster for males (37 individuals, 100%) (results not shown).

The density distribution plots indicate that the cluster differences evidentiated by the mixture analysis, are due

to divergence on both the PC1 and PC2 axes as the peaks of the distributions are clearly dislocated from each other on both axes (Fig. 1a–b). Note that the third peak is concealed by the other peaks, and therefore not visible, but see Table 1. It is also seen that the width of the density distribution peaks vary between clusters, which indicates differences in the variances of the PC1 and PC2 distributions (Fig. 1a–b).

Principal components analysis of the skull measurements of the East Greenland and Barents Sea populations

The PCA carried out on the covariance matrix derived from the skull measurements showed two convex polygons for both males and females, however, the two populations could generally not be distinguished by their scores on PC1 and PC2 due to the relatively large overlap (Fig. 2a–b). The first two PC axes (pooling the East Greenland and Barents Sea populations and holding the sexes separate) explained 80.3% (PC1) and 15.9% (PC2) of the total morphometric variation in males, and 81% (PC1) and 14.3% (PC2) of the total morphometric variation in females.
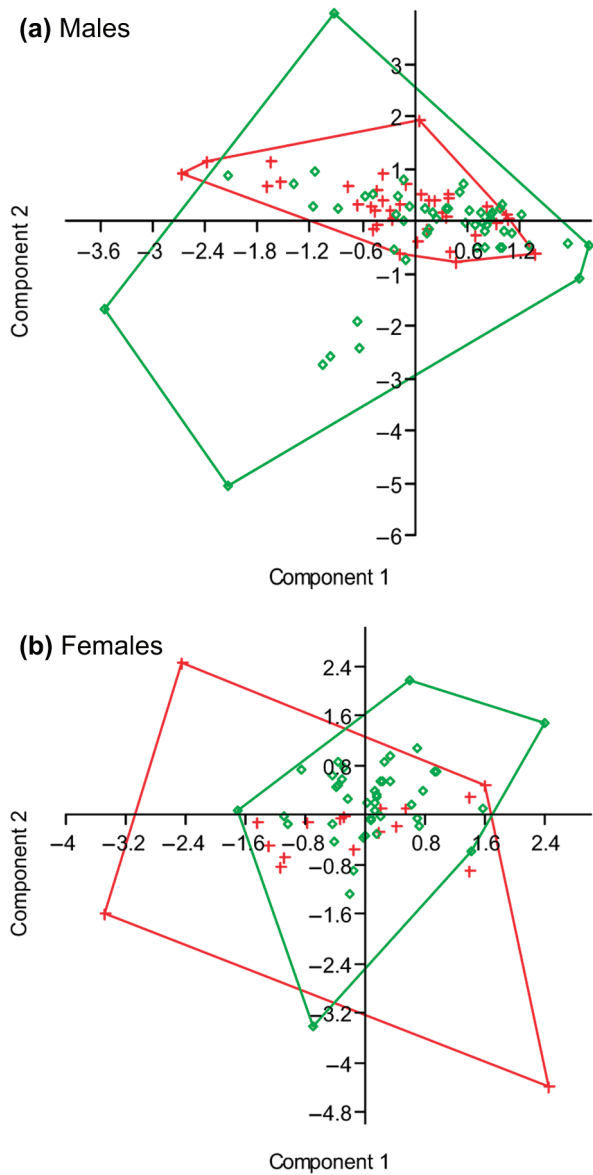
Principal components analysis of the clusters defined by mixture analysis

The PCA based on the clusters defined by the mixture analysis found one convex polygon for East Greenland males as well as females, and three convex polygons for both sexes of the Barents Sea population (Fig. 3a–b). Loadings on PC1 were all positive (females: 0.05–0.88, males: 0.04–0.69), thus summarizing the within-sample size variation (BOOKSTEIN 1989).

The three convex polygons for Barents Sea males and three convex polygons for Barents Sea females (Fig. 3) differed in both size (Fig. 1a–b, first axis) and shape (Fig. 1a–b, second axis). For the males (Fig. 3a), the East Greenland cluster (red polygon) overlapped two of the Barents Sea male bear clusters (green and blue) to a high extent, while only partially overlapping with the third Barents Sea convex polygons (purple). The PCA graph for female polar bears (Fig. 3b) shows the Barents Sea population as consisting of three convex polygons (blue, green and purple polygons) of which two were overlapping slightly (green and
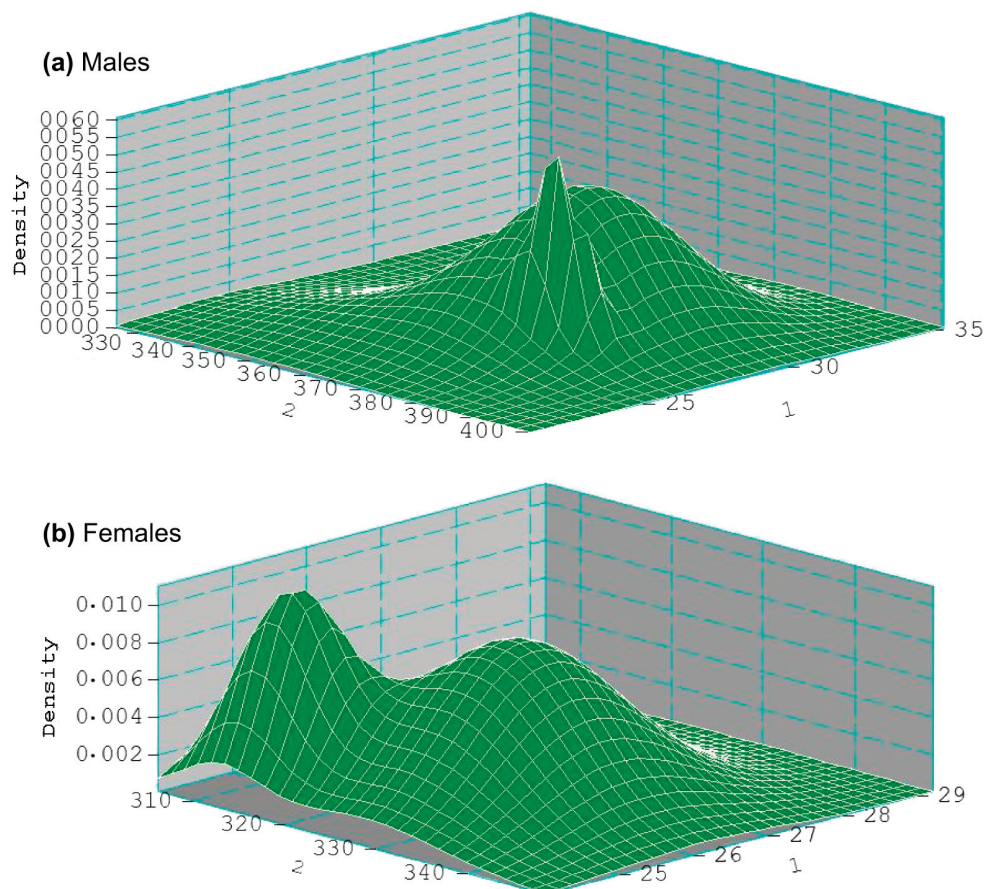
Fig. 1a–b. Density distribution of the scores on PC1 (1) and PC2 (2) axes of the convex polygons of Barents Sea polar bears identified by mixture analyses. Six convex polygons were identified in total; three for the males (**a**) and three for the females (**b**).

Fig. 2a–b. Plot of the first two axes (PC1 and PC2) from a principal component analysis for males (**a**) and female (**b**) polar bears of the East Greenland population (red line) and of the Barents Sea population (green line), based on a covariance matrix derived from skull measurements (see text for details).
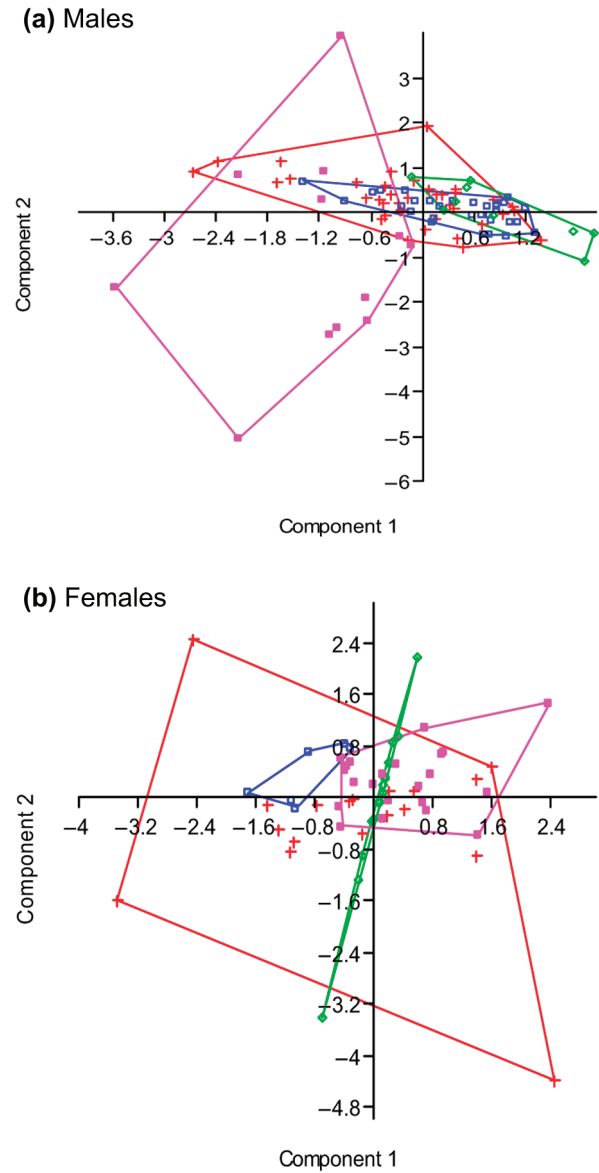
Fig. 3a–b. Plot of the first two axes (PC1 and PC2) from a principal component analysis for male (**a**) and females (**b**) polar bears of the East Greenland population cluster (red line) and of the Barents Sea subpopulation convex polygons (blue, green and purple lines) defined by mixture analyses.

purple). The East Greenland female population (Fig. 3b, red polygon) is shown as a convex polygon nearly completely overlapping all three Barents Sea convex polygons. For both sexes and especially for the Barents Sea population, the separation between the convex polygons appears to be primarily along the first axis (PC1, Fig. 3a–b). As the axes values are not independent of each other, this can only be evaluated qualitatively.

Multivariate analysis of variance of the skull measurements of the East Greenland and Barents Sea populations

The MANOVA showed no significant differences of multivariate means neither in males nor in females (p > 0.05, locations as well as sexes separate) with Wilk's lambda ranging from 0.87 to 0.91 and Pillai's trace ranging from 0.09 to 0.12. The non-significant results of the MANOVA confirm the graphical impression from

Fig. 2a and b where the convex polygons are largely overlapping.

Multivariate analysis of variance of the clusters defined by mixture analysis

The graphical representation of the results of the mixture analysis (Fig. 1a–b) shows a heterogeneous composition of the Barents Sea population. This is also confirmed by the MANOVA conducted on the clusters defined by mixture analysis, which shows significant differences in multivariate means in males (Wilk's lambda = 0.39, p < 0.001, Pillai trace = 0.72, p < 0.001), both between the three clusters of the Barents Sea population (3 out of 3 possible; Hotelling's test at the p < 0.05 level) and between each of the Barents Sea clusters and the East Greenland population (3 out of 3 possible; Hotelling's test at the $\alpha < 0.05$ level). No significant multivariate morphometric differentiation was found between the female polar bear populations of East Greenland and Barents Sea (Wilk's lambda = 0.72, p > 0.05, Pillai trace = 0.30, p < 0.05). Only one of the three Barents Sea female clusters was significantly different from the East Greenland female cluster (Hotelling's test: p < 0.05). However, when the MANOVA was conducted again, excluding the Greenland female population, morphometric differentiations between the three clusters of the Barents Sea population became significant (Wilk's lambda = 0.45, p < 0.001, Pillai trace = 0.58, p < 0.001, with 2 out of 3 Hotelling's tests significant at the p < 0.05 level), thus indicating internal differences between the clusters of the Barents Sea female bears.

DISCUSSION

Morphology and genetics

The mixture analyses revealed three clusters for Barents Sea males and three clusters for Barents Sea females. The number of individuals in the different clusters was not equally distributed; in both cases, the majority of the bears were collected in one of the three clusters; 50% of the female bears and 67% of the male bears, respectively. The East Greenland population consisted of one female and one male cluster. The East Greenland clusters overlapped the Barents Sea clusters to a high degree, especially with regards to the female polar bears. Despite there being some differences within and between the separate subpopulation clusters, the overall results indicate that many of the Barents Sea males and females are morphometrically similar to the East Greenland ones. This finding is in accordance with the results of Paetkau et al. (1999) who found virtually no genetic distance between the two populations although some differences in allele

frequency distributions existed. The results of Bechshøft et al. (2008a) also confirm the picture of especially the females of the two populations being particularly similar, in this case with regards to attainment of full size. Therefore, the results showed a heterogeneous composition of the Barents Sea population, despite there being no significant differences in their multivariate means (this is because the mixture analysis takes into account the covariance between the traits, not just the size, and therefore it is a more sensitive way of discriminating populations). These results are in accordance with Mauritzen et al. (2001), who found two kinds of movement patterns for females in the Barents Sea, as well as Aars et al. (2010), who found very little exchange between bears inhabiting the northern and southern parts of the Svalbard archipelago itself. However, Zeyl (2010) found that bears with different space use strategies (as defined by Mauritzen et al. 2001) did not belong to genetically differentiated groups; no sub-structure was found within the Barents Sea polar bears, which were thus assumed belonging to a single breeding unit. As was also found in the present study, the polar bears in East Greenland are thought to constitute a single population (Born in: IUCN PBSG 1995; Wiig 1995; Born et al. 2009). Satellite telemetry studies and movement of marked animals have also indicated that polar bears range widely along the coast of eastern Greenland and in the pack ice in the Greenland Sea and Fram Strait (Born et al. 1997; Wiig et al. 2003), and that the exchange between East Greenland and the Barents Sea population is minimal (Wiig 1995; Born et al. 1997; Wiig et al. 2003). Separate populations (East Greenland/Barents Sea) was further supported by Henrichsen and Sjøvold (in: IUCN PBSG 1986), Sonne et al. (2007b), and Bechshøft et al. (2008a, 2008b, 2008c). However, recent satellite telemetry results indicate that home ranges of bears from the East Greenland population overlap with those of bears from the Fram Strait (Born et al. 2009). This offers support for i.e. O'Gara et al. (in: IUCN PBSG 1980) and Larsen (1986), who hypothesize an exchange between the two populations, which would be in agreement with the present study.

Skull traits and size

In the present study, skull trait size (PC1) explained approx. 80% of the variation between the bears, whereas shape (PC2) defined approx. 15% of the morphometric variation, indicating some genetic and/or environmental differentiation. Hence, both environmental and genetic factors could have contributed to the observed skull differences between the two populations. It has been shown that the number of loci determining size variation is much smaller than the number of loci governing shape in mice

(WORKMAN et al. 2002; ROCHA et al. 2004; WOLF et al. 2006; LEAMY et al. 2008) and dogs (DRAKE and KLINGENBERG 2010). Therefore, it seems likely that shape variation is a better estimate of the genetic differences between populations and it could therefore be speculated that the observation of concomitant differentiation in both size and shape could indicate differences of the genetic pool.

Previous studies have shown that polar bears vary in size over their distribution range; BECHSHØFT et al. (2008a) found adult East Greenland polar bears to be larger in condylobasal length than bears from Barents Sea. This is in contrast to MANNING (1971), who suggested that Barents Sea polar bears might be larger than those from East Greenland, although he found no conclusive evidence in his data set to support the hypothesis. The Barents Sea polar bear population has previously been found to be the shorter in total body length when compared to other polar bear populations (DEROCHER and WIIG 2002). DEROCHER and WIIG (2002) found male, and especially female, polar bears from Barents Sea to be generally smaller in asymptotic body length and size plus lighter in weight than polar bears from a number of previously studied North American populations. Differences have also been found between the two populations with regards to fluctuating asymmetry (FA), both metric and meristic; Barents Sea bears had a higher incidence of FA than East Greenland bears in both cases (BECHSHØFT et al. 2008b, 2009). In addition to this, BECHSHØFT et al. (2008a) found skull parameters indicating that females of both localities matured at approximately the same age, whereas the Barents Sea males generally matured years later than their East Greenland peers. Furthermore, SONNE et al. (2007b) found that the size of baculum and uterus were significantly smaller in East Greenland polar bears than in their Barents Sea peers, as well as smaller than those from Canada (DYCK et al. 2004; SONNE et al. 2007b).

Considerations

We found a significant, and thus inherently higher, morphometric divergence in non-neutral quantitative traits between subpopulations (clusters) of East Greenland and Barents Sea polar bears than what PAETKAU et al. (1999) found using neutral molecular markers (microsatellites), which could suggest local adaptation. However, one must be aware that the Barents Sea polar bears analyzed by PAETKAU et al. (1999) were all collected at one locality (Hopen) in 1991 and as such not necessarily representative for the whole area whereas the skulls, on the other hand, have a broader sampling area and period.

Management

The East Greenland and the Barents Sea polar bear populations are managed as separate MUs (IUCN PBSG 2010). The current status of the East Greenland polar bear population is unknown as no population inventory has been conducted to date (IUCN PBSG 2010). Quotas were implemented for the polar bear hunt in East Greenland in 2006 (2006–2010: 30 animals year^{-1} in Ittoqqortoormiit area, 20 animals year^{-1} in Ammassalik, and four in southwest Greenland and in 2011: 35 animals year^{-1} in Ittoqqortoormiit area, 25 animals year^{-1} in Ammassalik, and four in southwest Greenland; Anonymous 2006, 2010, 2011). The Barents Sea polar bear population has been subject to extensive harvest 1870–1973 (LØNØ 1970; LARSEN 1986), but all hunting in the area was banned in 1973 and the population may now be showing signs of recovery (DEROCHER 2005; AARS et al. 2009). ZEYL et al. (2009) suggest that population recovery in the Barents Sea population, after the long history of hunting, occurred not only through long-distance immigration, but maybe even more so by families using surrounding vacant areas. MAURITZEN et al. (2001, 2002) found two kinds of movement patterns for polar bears in the Barents Sea based on satellite telemetry. A subpopulation structure in the Barents Sea population was also indicated from the present analyses of morphometric data. On the other hand, ZEYL (2010) did not detect any genetic structure in this population. However, if a substructure does indeed exist in the population this should be considered in relation to future management.

Conclusions

The present study shows the comparison of differences in size and shape of East Greenland and Barents Sea polar bear skulls by application of a multivariate approach, thereby characterizing the degree of adaptive genetic variation between the two populations by using morphometrics as an indicator of environmental and/or genetic differences. Overall, results indicate that many Barents Sea polar bears are morphometrically similar to the East Greenland ones, suggesting an exchange of individuals and genes between the two populations. However, despite the flow between them, both populations seem to be adapted to different environmental conditions. Furthermore, a subpopulation structure in the Barents Sea population was also indicated from the present analyses of morphometric data.

Even though mixture analysis on biological data has a long history (PEARSON 1894), few biological studies have been carried out (PERTOLDI et al. 2006, 2009). We find it advantageous because it performs an unbiased analysis of the data without a priori expectations, unlike many other

approaches, which test the difference among pre-specified groups, which may or may not be real. Since the method does not use geographical information as an input, the grouping of a significant number of individuals from the same localities in the same cluster gives strong evidence of geographic differentiation and therefore there the mixture analysis should be considered a very useful complementary tool for the definition of MUs and ESUs.

REFERENCES

Aars, J., Marques, T. A., Buckland, S. T. et al. 2009. Estimating the Barents Sea polar bear subpopulation size. – Mar. Mammal Sci. 25: 35–52.

Aars, J., Derocher, A. E., Jenssen, B. M. et al. 2010. Polar bear research in Norway, 2005–2009. – In: Obbard, M. E., Thiemann, G. W., Peacock, E. et al. (eds), Polar bears. Proc. 15th Working Meeting of the IUCN/SSC Polar Bear Specialist Group, Copenhagen, Denmark, 29 June–3 July 2009. IUCN, pp. 157–163.

Andersson, M. 1994. Sexual selection. – Princeton Univ. Press.

Anonymous 2006. Kvote for fangst af hvalros og isbjørn for kvoteårene 2007–2009. Tusagassiorf nnut nalunaarut/Pressemeddelelse 18. december 2006. – Namminersornerullutik, Oqartussat Aalisarnermut, Piniarnermut Nunalerinermullu Naalakkersuisoq/Grønlands Hjemmestyre Landstyremedlemmet for Fiskeri, Fangst og Landbrug.

Anonymous 2010. Midlertidige kvoter for fangst af isbjørn og hvalros for 2010. Website visited 29 Nov. 2010. < http://mipi.nanoq.gl/sitecore/content/Websites/nanoq/Emner/Landsstyre/Departementer/Departement_for_fiskeri/Nyhedsforside/Nyheder_fra_dep_fiskeri/2009/12/MidlertidigkvoterIsbjornMoskus2010.aspx >

Anonymous 2011. Isbjørnekvoter 2011. Website visited 10 Feb. 2011. < http://dk.nanoq.gl/Emner/Landsstyre/Departementer/Departement_for_fiskeri/Nyhedsforside/Nyheder_fra_dep_fiskeri/2011/01/isbjoerne_kvoter_2011.aspx >

Bechshøft, T. Ø., Sonne, C., Rigét, F. F. et al. 2008a. Differences in growth, size and sexual dimorphism in skulls of East Greenland and Barents Sea polar bears (*Ursus maritimus*). – Polar Biol. 31: 945–958.

Bechshøft, T. Ø., Wiig, Ø., Sonne, C. et al. 2008b. Temporal and spatial variation in metric asymmetry in skulls of polar bears (*Ursus maritimus*) from East Greenland and Barents Sea. – Ann. Zool. Fenn. 45: 15–31.

Bechshøft, T. Ø., Rigét, F. F., Wiig, Ø. et al. 2008c. Fluctuating asymmetry in metric traits: a practical example of calculating asymmetry, measurement error and repeatability. – Ann. Zool. Fenn. 45: 32–38.

Bechshøft, T. Ø., Rigét, F. F., Sonne, C. et al. 2009. Skull foramina asymmetry in East Greenland and Barents Sea polar bears (*Ursus maritimus*) in relation to stressful environments. – Ann. Zool. Fenn. 46: 181–192.

Bookstein, F. L. 1989. Size and shape – a comment on semantics. – Syst. Zool. 38: 173–180.

Born, E. W., Wiig, Ø. and Thomassen, J. 1997. Seasonal and annual movements of radio-collared polar bears (*Ursus maritimus*) in northeast Greenland. – J. Mar. Syst. 10: 67–77.

Born, E. W., Dietz, R., Wiig, O. et al. 2009. Polar bear *Ursus maritimus*. – In: Boertmann, D., Mosbech, A., Schiedek, D. et al. (eds), The western Greenland Sea. A preliminary strategic environmental impact assessment of hydrocarbon activities in the Kanumas East area. Natl Environ. Res. Inst., Aarhus Univ., Denmark. NERI Tech. Rep. no. 719. < www.dmu.dk//Pub/FR719.pdf >

Chase, K., Carrier, D. R., Adler, F. R. et al. 2002. Genetic basis for systems of skeletal quantitative traits: principal component analysis of the canid skeleton. – Proc. Natl Acad. Sci. USA 99: 9930–9935.

deGuia, A. P. O. and Saitoh, T. 2007. The gap between the concept and definitions in the evolutionary significant unit: the need to integrate neutral genetic variation and adaptive variation. – Ecol. Res. 22: 604–612.

Derocher, A. E. 2005. Population ecology of polar bears at Barents Sea, Norway. – Popul. Ecol. 47: 267–275.

Derocher, A. E. and Wiig, Ø. 2002. Postnatal growth in body length and mass of polar bears (*Ursus maritimus*) at Barents Sea. – J. Zool. 256: 343–349.

Derocher, A. E., Andersen, M. and Wiig, Ø. 2005. Sexual dimorphism of polar bears. – J. Mamm. 86: 895–901.

Dietz, R., Heide-Jørgensen, M. P. and Teilmann, J. et al. 1991. Age determination in European Harbour seals *Phoca vitulina* L. – Sarsia 76: 17–21.

Drake, A. G. and Klingenberg, C. P. 2010. Large-scale diversification of skull shape in domestic dogs: disparity and modularity. – Am. Nat. 175: 289–301.

Dyck, M. G., Bourgeois, J. M. and Miller, E. H. 2004. Growth and variation in the bacula of polar bears (*Ursus maritimus*) in the Canadian Arctic. – J. Zool. 264: 105–110.

Endler, J. A. 1986. Natural selection in the wild. – Princeton Univ. Press.

Hammer, Ø., Harper, D. A. T. and Ryan, P. D. 2004. PAST – Palaeontological statistics. ver. 1.21. < http://folk.uio.no/ohammer/past >

Hensel, R. J. and Sorensen, F. E. 1980. Age determination of five polar bears. – Int. Conf. on Bear Res. Manage. 4: 93–100.

IUCN PBSG (Polar Bear Specialist Group) 1980. Polar bears. Proc. 7th working meeting of the IUCN Polar Bear Specialist Group, 30 January–1 February 1979, Copenhagen, Denmark and Proc. 6th working meeting of the IUCN Polar Bear Specialist Group, 7–10 December 1976, Gland, Switzerland. IUCN, Gland, Switzerland.

IUCN PBSG (Polar Bear Specialist Group) 1986. Polar bears. Proc. 9th working meeting of the IUCN/SCC Polar Bear Specialist Group, 9–11 August 1985, Edmonton, Alberta, Canada. IUCN, Gland, Switzerland.

IUCN PBSG (Polar Bear Specialist Group) 1995. Polar Bears. Proc. 11th working meeting of the IUCN/SSC Polar Bear Specialist Group. – Occ. Pap. of IUCN/SSC no. 10. IUCN, Gland Switzerland and Cambridge, UK.

IUCN PBSG (Polar Bear Specialist Group) 2006. Polar Bears. Proc. 14th working meeting of the IUCN/SSC Polar Bear Specialist Group, 20–24 June 2005, Seattle, Washington, USA. IUCN, Gland, Switzerland and Cambridge, UK.

IUCN PBSG 2010. – In: Obbard, M. E., Thiemann, G. W., Peacock, E. et al. (eds), Polar bears, Proc. 15th Working meeting of the IUCN/SSC Polar bear Specialist Grp, Copenhagen, Denmark, 2009. IUCN.

Kawecki, T. J. and Ebert, D. 2004. Conceptual issues in local adaptation. – Ecol. Lett. 7: 1225–1241.

Larsen, T. 1986. Population biology of the polar bear (*Ursus maritimus*) in the Barents Sea area. – Norsk Polarinst. Skrifter 184.

Leamy, L. J., Klingenberg, C. P., Sherratt, E. et al. 2008. A search for quantitative trait loci exhibiting imprinting effects on mouse mandible size and shape. – Heredity 101: 518–526.

Lønø, O. 1970. The polar bear (*Ursus maritimus* Phipps) in the Barents Sea area. – Norsk Polarinst. Skrifter 149.

Lynch, M. 1996. A quantitative-genetic perspective on conservation issues. – In: Avise, J. C. and Hamrick, J. L. (eds), Conservation genetics. Case histories from nature. Chapman and Hall, pp. 471–501.

Manning, T. H. 1971. Geographical variation in the polar bear *Ursus maritimus* Phipps. – Can. Wildlife Serv. Rep. Ser. 13: 4–27.

Marcus, L. F. 1990. Traditional morphometrics. – In: Rohlf, F. J. and Bookstein, F. L. (eds), Proc. Michigan Morphometrics Workshop. Univ. of Michigan Zool. Mus., Ann Arbor, MI.

Marriott, F. H. C. 1975. Separating mixtures of normal distribution. – Biometrics 31: 767–769.

Mauritzen, M., Derocher, A. E. and Wiig, Ø. 2001. Space use strategies of female polar bears in a dynamic sea ice habitat. – Can. J. Zool. 79: 1704–1713.

Mauritzen, M., Derocher, A. E., Wiig, Ø. et al. 2002. Using satellite telemetry to define spatial population structure in polar bears in the Norwegian and western Russian Arctic. – J. Appl. Ecol. 39: 79–90.

McLachlan, G. J. and Peel, D. 1998. Robust cluster analysis via mixtures of of multivariate t-distributions. – In: Amin, A. Dori, D., Pudil, P. et al. (eds), Lecture notes in computer science, Vol. 1451, Springer, pp. 658–666.

Merilä, J. and Crnokrak, P. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. – J. Evol. Biol. 14: 892–903.

Moritz, C. 1994. Defining 'evolutionary significant units' for conservation. – Trends Ecol. Evol. 9: 373–375.

Paetkau, D., Amstrup, S. C., Born, E. W. et al. 1999. Genetic structure of the world's polar bear populations. – Mol. Ecol. 8: 1571–1584.

Pearson, K. 1894. Contributions to the mathematical theory of evolution. – Philos. Trans. R. Soc. A 185: 71–110.

Pertoldi, C., García-Perea, R., Godoy, J. A et al. 2006. Morphological consequences of range fragmentation and population decline on the endangered Iberian lynx (*Lynx pardinus*). – J. Zool. 268: 73–86.

Pertoldi, C., Bijlsma, R. and Loeschcke, V. 2007. Conservation genetics in a globally changing environment: present problems, paradoxes and future challenges. – Biodivers. Conserv. 16: 4147–4163.

Pertoldi, C., Sonne, C., Dietz, R. et al. 2009. Craniometric characteristics of polar bear skulls from two periods with contrasting levels of industrial pollution and sea ice extent. – J. Zool. 279: 321–328.

Rocha, J. L., Eisen, E. J., Van Vleck, L. D. et al. 2004. A large-sample QTL study in mice, II: Body composition. – Mamm. Genome 15: 100–115.

Rosing-Asvid, A., Born, E. W. and Kingsley, M. C. S. 2002. Age at sexual maturity of males and timing of the mating - season of polar bears (*Ursus maritimus*) in Greenland. – Polar Biol. 25: 878–883.

Sonne, C., Rigét, F. F., Dietz, R. et al. 2007a. Skull pathology in East Greenland and Barents Sea polar bears (*Ursus maritimus*) during 1892 to 2002 in relation to organochlorine pollution. – Sci. Total Environ. 372: 554–561.

Sonne, C., Dietz, R., Born, E. W. et al. 2007b. Spatial and temporal variation in size of polar bear (*Ursus maritimus*) sexual organs and its use in pollution and climate change studies. – Sci. Total Environ. 387: 237–246.

Waples, R. S. 1991. Pacific salmon, *Oncoryhnchus* spp., and the definition of species under the Endangered Species Act. – Mar. Fish. Rev. 53: 11–22.

Wiig, Ø. 1995. Distribution of polar bears (*Ursus maritimus*) in the Barents Sea area. – J. Zool. 237: 515–529.

Wiig, Ø., Born, E. W. and Pedersen, L. T. 2003. Movements of female polar bears (*Ursus maritimus*) in the East Greenland pack ice. – Polar Biol. 26: 509–516.

Wolf, J. B., Pomp, D., Eisen, E. J. et al. 2006. The contribution of epistatic pleiotropy to the genetic architecture of covariation among polygenic traits in mice. – Evol. Dev. 8: 468–476.

Workman, M. S., Leamy, L. J., Routman, E. J. et al. 2002. Analysis of QTL effects on the size and shape of mandibular molars in mice. – Genetics 160: 1573–1586.

Zeyl, E. 2010. – PhD thesis, Natl Centre for Biosystematics, Nat. Hist. Mus., Fac. Math. Nat. Sci., Univ. of Oslo, Norway.

Zeyl, E., Aars, J., Ehrich, D. et al. 2009. Families in space: relatedness in the Barents Sea population of polar bears (*Ursus maritimus*). – Mol. Ecol. 18: 735–749.