Analysis of mitochondrial control region nucleotide sequences from Baffin Bay beluga, (*Delphinapterus leucas*): detecting pods or sub-populations?

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ABSTRACT

We report the results of an analysis of the variation in the nucleotide sequence of the mitochondrial control region obtained in 218 samples collected from belugas, *Delphinapterus leucas*, around the Baffin Bay. We detected multiple instances of significant heterogeneity in the distribution of genetic variation among the analyzed mitochondrial control region sequences on a spatial as well as temporal scale indicating a high degree of maternal population structure. The detection of significant levels of heterogeneity between samples collected in different years but within the same area and season was unexpected. Re-examination of earlier results presented by Brown Gladden and coworkers also revealed temporal genetic heterogeneity within the one area where sufficient (n>15) samples were collected in multiple years. These findings suggest that non-random breeding and maternally directed site-fidelity are not the sole causes of genetic heterogeneity among belugas but that a matrilineal pod structure might cause significant levels of genetic heterogeneity as well, even within the same area. We propose that a maternal pod structure, which has been shown to be the cause of significant genetic heterogeneity in other odontocetes, may add to the overall level of heterogeneity in the maternally inherited DNA and hence that much of the spatial heterogeneity observed in this and previous studies might be attributed to pod rather than population structure. Our findings suggest that it is important to estimate the contribution of pod structure to overall heterogeneity before defining populations or management units in order to avoid interpreting heterogeneity due to sampling of different pods as different populations/management units.

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INTRODUCTION

Belugas, *Delphinapterus leucas*, constitute an essential part of the local diet and economy in Greenland. Recent abundance estimates have indicated a substantial decline in abundance between 1981 and 1999 among belugas that winter in the area from Disko Bay area and southward (Heide-Jørgensen and Acquarone 2002). In contrast, no such decline has been detected among belugas that winter in the North Water in the northern part of the Baffin Bay (Innes and Stewart 2002). The difference in the rate of decline among belugas in the Disko Bay and North Water could be taken to indicate the existence of at least two different populations in the northern and eastern Baffin Bay area, each of which are likely to require different management schemes (Innes and Stewart 2002).

From the seasonal timing of catches of belugas off West Greenland it appears there is a major southbound migration during the autumn passing through Qaanaaq and Upernavik (Fig. 1) municipality during late September to early October, wintering in the Disko Bay where belugas arrive in November (Heide-Jørgensen 1994). During the winter (January through March) belugas are also observed and harvested further south off West Greenland in the Sisimiut and Maniitsoq municipalities and sightings have been reported as far south as off Paamiut. The sighting and catch records indicate that most belugas remain in the area between Maniitsoq and Disko Island throughout the winter until mid-May when a northbound migration takes place. The belugas that occasionally are harvested at the ice edge in the Upernavik municipality during spring are thus presumably from this northbound spring migration from the Disko Bay area. In addition to belugas observed in the Disko Bay and off Sisimiut, belugas are also occasionally sighted in the northern Baffin Bay at Qaanaaq municipality, during the summer in fjords and off the ice edge in winter. A limited number of belugas are harvested in Qaanaaq municipality during the winter.

The above mentioned sighting and catch records indicate a minimum of two separate winter (and thus presumably breeding) grounds in the general Baffin Bay area for belugas; the aforementioned area between Maniitsoq and the Disko Bay as well as the North Water, a polynya west of Qaanaaq. At present it is not known where belugas that summer in the fjords off southeastern Baffin Island winter, although the results obtained from belugas instrumented with satellite-linked radio transmitters did not suggest a migration in the direction of West Greenland (Heide-Jørgensen and Richard, un-

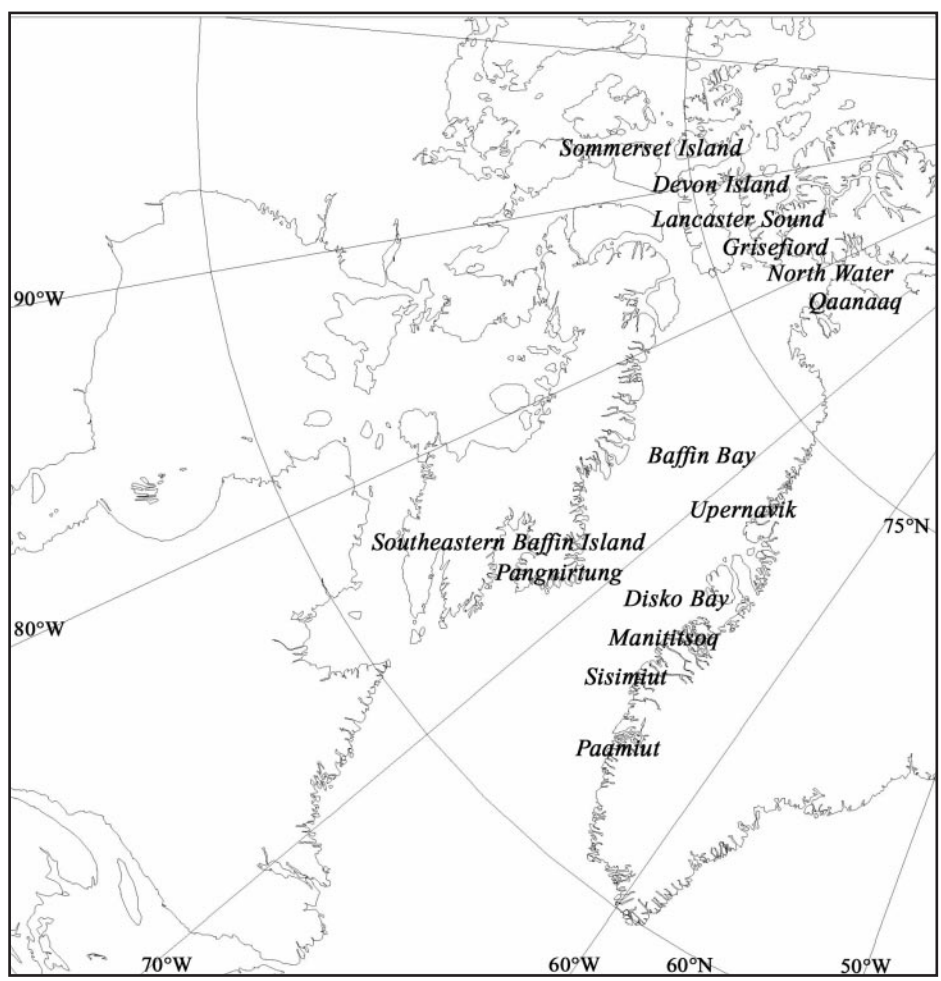

Fig.1
The geographic position of localities mentioned in the text.

published data). It might be that these whales winter in the pack ice off southeast Baffin Island.

During the summer few belugas are observed off West Greenland, whereas large herds of belugas congregate in estuaries at Somerset Island in the Canadian High Arctic. A more detailed insight into the migration pattern of these belugas has been obtained by use of satellite tracking. Twenty-four belugas instrumented with radio satellite-linked transmitters at Somerset Island were detected moving eastward out into Lancaster Sound in mid-September. Of the 24 instrumented belugas, 21 individuals were tracked until October 10th at which time they all had arrived at the North Water (Richard *et al*. 2001) and not further south off West Greenland as expected if they were wintering in the Disko Bay area. Hence, these results indicate that the radio-instrumented belugas, which summer in the Canadian High Arctic, did not constitute part of the belugas migrating south along West Greenland during the autumn. However, one instrumented whale did migrate from the Canadian High Arctic to West Greenland and arrived in Qaanaaq and Upernavik municipality during early October, at the peak of the autumn southbound migration. Radio contact was lost with this single individual in late October, just west of Disko Island. This observation suggests the possible mixture of individuals from different winter breeding grounds during the summer in the Canadian High Arctic.

The results from the instrumented belugas showed that some belugas that summer in the Canadian High Arctic might winter in the area south of Disko Bay. Although the proportion of such individuals appeared low among the belugas tracked with satellite-linked radio transmitters to date, their proportion might be higher in other parts of the Canadian High Arctic where no belugas have yet been instrumented with radio tags. In addition, the results from the satellite radio transmitter experiments confirmed the occurrence and timing of an autumn migration southward along the West Greenland coast. The tagging experiments have also corroborated the notion that the winter population of belugas in the North Water may be separate from those wintering south of the Disko Bay.

The fact that the spatial distribution of belugas is recurrent in space within each season suggests a temporally and spatially consistent migration pattern of individual belugas utilising the same areas in consecutive years. Brown Gladden *et al*. (1997) and O'Corry-Crowe *et al*. (1997) reached the same conclusion from analyses of the distribution of genetic variation in the nucleotide sequence of the mitochondrial control region among belugas sampled across High and Low Arctic North America. Both studies detected a high degree of genetic structure among samples in the maternally transmitted mitochondrial genome among summer grounds of belugas. A subsequent analysis of Mendelian inherited microsatellite loci among Canadian belugas (Brown Gladden *et al*. 1999) found a less pronounced population genetic structure within larger breeding populations. The finding of a differential degree of divergence at mitochondrial and nuclear loci is consistent with the notion of a maternal directed population structure. The repeated occurrence of belugas on specific summer and winter grounds prompted Brown Gladden *et al*. (1997) to suggest that maternally directed site-fidelity to specific summer and winter grounds was the driving force behind the observed maternal population genetic structure.

Multivariate analyses of organochlorine profiles in beluga blubber samples also revealed significant differences between West Greenland and a number of Canadian locations (Innes *et al*. 2002). Their study also suggested differences in organochlorine profiles among belugas within the West Greenland area. As for the mitochondrial genome, organochlorines are likely to be transferred maternally (via lactation) and thus these results could be taken as corroborating the results of the genetic studies pointing at a matrilineal population structure.

On a more detailed scale, little is known regarding the pod structure of belugas. Data collected from the autumn harvest in West Greenland indicates the existence of pods consisting of mature females accompanied by younger animals of both sexes in the Upernavik municipality (Heide-Jørgensen and Teilmann 1994). Mature, adult males were absent from this autumn harvest but present in the winter catches from

Disko Bay and south. Males appear to travel in separate pods consisting of mature males only (Heide-Jørgensen, unpublished data).

Overall, the available data suggests a pod structure similar to that of narwhals, *Monodon monoceros*, where mature males and females seem only to mix on the winter grounds during the breeding season (Smith *et al*. 1994, Koski and Davis 1994).

The continued harvest of belugas along the entire coast of West Greenland coupled with the substantial decline detected among the belugas wintering in and south off Disko Bay necessitates an informed basis for an appropriate management scheme, where the population identity of belugas observed off West Greenland constitutes a key issue. The objective of the present study was to employ genetic analyses of the mitochondrial control region to gain further insight into the population structure of belugas off West Greenland to aid the delineation of biologically relevant management units.

MATERIAL AND METHODS

Samples

Samples were collected on-site during aboriginal subsistence hunting either by local hunters in the form of the lower jaw or by biologists from the Greenland Institute of Natural Resources. The lower jaws were subsequently sent frozen to Copenhagen (Denmark) where skin samples were collected. Samples were stored at minus 20˚ C until analysis. In most instances genomic DNA was extracted from the skin, although in some cases muscle or kidney tissue was used instead in cases when skin was not available and for samples collected from whole animals.

Laboratory analyses

Total-cell DNA was extracted from the tissue samples using standard protocols (Maniatis *et al*. 1982) of cell-lysis by addition of 10% SDS and overnight digestion of proteins by Proteinase K at 65˚ C. The nucleic acids were extracted by repeated phenol-chloroform extraction and precipitated with 96% ethanol. The precipitated nucleic acids were re-suspended in 1xTE (Maniatis *et al*. 1982).

The nucleotide sequence of the 3'end of mitochondrial control region was determined by direct sequencing of PCR (Polymerase Chain Reaction, Mullis and Faloona 1987) amplification products as described in Palsbøll *et al*. (1997).

The sex of each sample was determined either by morphology when possible or by molecular means, following the protocol for odontocetes outlined in Bérubé and Palsbøll (1996) or Palsbøll *et al*. (1992).

Data analyses

The degree of genetic differentiation between sample partitions was estimated as K_{ST} (Hudson 1992, Hudson *et al*. 1992), which is basically the proportion of the nucleotide diversity in the overall sample, which is attributable to the partition of samples. The probability of the observed K_{ST} under a model of panmixis was estimated from 1,000 Monte Carlo permutations as described by Hudson *et al*. (1992).

Homogeneity tests were first conducted between males and females collected the same year within each sample area. In the absence of any significant heterogeneity male and female samples from each year were combined in subsequent tests. Further homogeneity tests were undertaken employing two different schemes to partition samples; geographical proximity or the assumed migration pattern. In the former scheme samples from the same sampling area were compared across years and if no significant heterogeneity was detected they were then combined and compared with the closest neighbouring sampling areas, and so on. The latter sampling scheme attempted to take the different sampling seasons as well as the presumed migratory destinations into account. For example, samples collected in the autumn from Upernavik municipality were first compared with those from the Disko Bay area (presumably the wintering ground for the migrating belugas that pass Upernavik) and subsequently with samples from other populations, such as those collected in Qaanaaq during the winter.

When multiple simultaneous estimations of significance were undertaken the obtained *P*-values were corrected to a table-wide value of $\alpha = 0.05$ by sequential Bonferroni corrections as outlined by Rice (1989).

A genealogy, rooted with the homologous mitochondrial control region sequence from narwhal, was estimated from the beluga haplotypes detected in the study. A total of 1,000 bootstrap samples were generated using the PHYLIP 3.52c computer package (Felsenstein 1993). For each of the 1,000 bootstrap samples Kimura's two-parameter distances (Kimura 1980) were estimated between haplotypes and genealogies estimated using the Neighbour-joining Method (Saitou and Nei 1987) with a transition:transversion ratio of 20 and the jumple option on. The bootstrap support for each node was then derived from a majority-rule consensus genealogy.

RESULTS

Between 185 and 278 nucleotides at the beginning of the mitochondrial control region were sequenced in a total of 218 specimens from West Greenland and eastern Canada. When using a cut-off at 244 nucleotides the data set was reduced to a total of 195 specimens, which constitute the data used for the present study (Tables 2 and 3).

Of the 195 samples, 167 were from West Greenland covering the range from Qaanaaq in the North to Sisimiut in the south. The remaining 28 samples were collected in the eastern

Table 1
Polymorphic sites among the first 244 nucleotides of the part of the mitochondrial control region sequenced in this study

Haplotype	8	18	38	42	43	68	69	70	71	195	197	101	115	129	149	291	292	211	214	23	32
DL001	A	C	A	C	G	A	A	T	A	C	C	T	A	T	T	A	T	G	C	T	C
DL002	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
DL003	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	G	.	.	.	.	.
DL004	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
DL005	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.
DL006	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	G	.	.	.	.	.
DL007	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.	.	.	.
DL008	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	T	.	.
DL009	G	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	C	A	T	.	T
DL010	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	C	.
DL011	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	C	.	.	.	.
DL012	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	C	.	.	.	T
DL013	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	G	.	.	.	.	.
DL014	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
DL015	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
DL016	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	T
DL017	.	.	.	G	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
DL018	.	.	.	.	.	G	G	A	G	.	.	.	.	.	.	G	.	.	.	.	.
DL019	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.

Note: The consensus sequence (DL001) is listed in its entire length in Figure 2.

```
1           11          21          31          41          51
AAAAAGGATT  TATTGTACAA  TAACAAACCA  CTACAGTACT  ACGTCAGTAT  TAAATAA

61          71          81          91          101         111
TATTTCCAAT  ACATTTTACT  GTGACTATTG  CATACCCTTA  TACACACACC  ATTAAAT

121         131         141         151         161         171
AGTCTTTCTT  TATAAATATT  CATATACATA  TATACTATGT  ATTATTGTGC  ATTCATT

181         191         201         211         221         231
TTTCCATACG  ATCAGTTAAA  GCTCGTATTA  GATCTTATTA  ATTTTACAAA  TCACATA

241
TGCA
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***Fig. 2**. The consensus (DL001, Table 1) nucleotide sequence of the mitochondrial control region.*

Table 2. The number of the different mitochondrial control region haplotype sequences detected in this study listed per year and sampling area off West Greenland

Sampling region	Mitochondrial control region haplotypes as defined in Table 1															
	DL001	DL002	DL003	DL004	DL005	DL006	DL007	DL008	DL009	DL010	DL011	DL012	DL013	DL015	DL016	Total
Sisimiut & Maniitsoq																
1993	2	6	-	-	-	1	-	-	-	-	-	-	1	1	-	11
1994	9	18	-	1	1	-	2	-	-	1	-	-	-	-	-	32
Total	11	24	-	1	1	1	2	-	-	1	-	-	1	1	-	43
Disko Bay																
1986	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
1989	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2
1990	2	6	-	-	2	-	-	-	-	-	-	-	-	-	-	10
1992	4	3	-	-	-	-	-	-	-	-	-	-	-	-	-	6
1993	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	5
Total	11	13	-	-	2	-	-	-	-	-	-	-	-	-	-	26
Upernavik																
1985	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	4
1989	2	4	-	-	1	-	-	1	-	-	-	-	1	-	-	9
1990	1	4	-	-	-	1	-	-	-	-	-	-	1	-	-	7
1992	15	13	-	-	-	1	1	1	1	-	-	-	-	-	-	32
1993	16	12	1	-	1	-	-	-	-	-	1	-	-	-	-	31
Total	34	37	1	-	2	2	1	2	1	-	1	-	2	-	-	83
Qaanaaq																
1984	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
1990	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	2
1993	5	4	1	-	-	-	-	-	-	-	-	1	-	-	1	12
Total	5	5	1	-	-	-	-	-	-	-	-	3	-	-	1	15
Overall	61	79	2	1	5	3	3	2	1	1	1	3	3	1	1	167

Table 3.
The number of the different mitochondrial control region haplotype sequences detected in this study listed per year and sampling area in eastern Canada.

Sampling region	Mitochondrial control region haplotypes as defined in Table 1						
	DL001	DL002	DL014	DL017	DL018	DL019	Total
Southeast Baffin Bay							
1986	8	-	1	-	1	1	11
1991	1	-	-	-	-	-	1
1992	1	-	1	-	-	-	2
Total	10	-	2	-	1	1	14
Grise Fiord/Devon Island							
1985	1	-	-	1	-	-	2
1987	2	3	1	-	-	-	6
1991	-	-	-	-	-	-	0
1995	4	2	-	-	-	-	6
Total	7	5	1	1	-	-	14
Overall	17	5	3	1	1	1	28

Canadian Arctic; at Devon Island, Grise Fjord as well as southeastern Baffin Bay. A total of 21 polymorphic sites were detected defining 19 different haplotypes (Table 1). Of the 19 substitutions 2 were transversions. The estimated genealogy detected only a few nodes with bootstrap values above 50%, revealing no or little phylogenetic signal among the detected haplotypes (Fig. 3).

The nucleotide diversity for the overall sample was estimated at .0043 (SE .00048), ranging from .0031 (SE .00073) in Upernavik municipality to .0092 (SE .0018) in Qaanaaq municipality. The estimated degree of nucleotide diversity was thus significantly higher among the samples collected in the Qaanaaq municipality relative to most other sampling areas (see Table 4).

No significant levels of genetic heterogeneity were detected between sexes or between years within the same sampling area apart from the Upernavik municipality (Table 4). Spatially or temporally adjacent samples between which no significant level of heterogeneity was detected were pooled in subsequent homogeneity tests. Following this procedure six partitions of samples were determined as significantly different within West Greenland: the Qaanaaq municipality, the Upernavik municipality prior to 1992, the Upernavik municipality in 1992 and 1993, and finally the Disko Bay/ Maniitsoq/ Sisimiut region. We detected a significant level of genetic heterogeneity in all but two of six

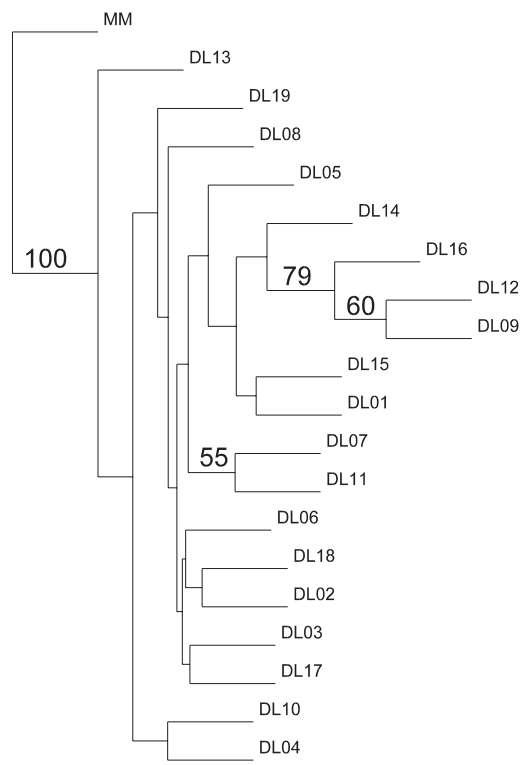

Fig. 3.
The genealogy estimated from the 19 mitochondrial control region haplotypes detected in this study, rooted with the homologous nucleotide sequence from narwhal (Palsbøll et al. 1997).

Note.
MM denotes narwhal. DL001-DL019 denotes the beluga mitochondrial control region haplotype sequences listed in Table 1.

Table 4
The degree of genetic variation at each homogenous sample area estimated as the nucleotide diversity as well as estimates of the degree of genetic divergence estimated between those sampling areas

Sampling area	Sampling area			
	Sisimiut/Maniitsoq /Disko	Upernavik pre 1992	Upernavik 1992 & 1993	Qaanaaq
Sisimiut/Maniitsoq/Disko	.0032 (.00039)	.0094 (<.12)	.014 (<.037)	**.063 (<.003)**
Upernavik pre 1992		.0031 (.00073)	**.046 (<.006)**	**.11 (<.00001)**
Upernavik 1992 & 1993			.0039 (.00092)	**.035 (<.017)**
Qaanaaq				.0092 (.0018)

Note. The estimated nucleotide diversity (Nei and Li 1979) within each sampling area is listed in the diagonal with standard errors in parentheses. The degree of genetic divergence between sampling areas expressed as K_{ST} (Hudson *et al*. 1992) with the probability of the observed value if both samples were collected from one population, estimated by permutations (Hudson *et al*. 1992). Bold numbers are *P*-values that are significant at the table-wide level (α=0.05) after sequential Bonferroni corrections (Rice 1989).

pairwise comparisons between these sampling regions. After sequential Bonferroni corrections neither of the Upernavik samples were heterogeneous to those from Disko Bay/Maniitsoq/ Sisimiut although one *P*-value is marginal (*P*<.034). Thus, as has been shown previously (Brown Gladden *et al*. 1997, O'Corry-Crowe *et al*. 1997), the degree of genetic heterogeneity appears high even between close geographical areas. However, in the present study we detected significant levels of genetic heterogeneity between years *within* the same geographical region.

Among the Canadian sampling localities significant levels of genetic heterogeneity were detected between the southeastern Baffin Island and the combined Devon Island and Grise Fjord sample. Although none of the Canadian-West Greenland homogeneity tests were significant at the .05 level after sequential Bonferroni corrections, three of eight *P*-values were estimated at less than .05 (Table 5). The highest levels of genetic heterogeneity between West Greenland and Canadian sampling localities were estimated between the most distant sampling localities.

Attempts to partition samples according to the putative breeding population and migration patterns did not improve the consistency in terms of genetic divergence within and among "subpopulations".

DISCUSSION

High degree of genetic heterogeneity among Baffin Bay belugas

In agreement with previous analyses of the distribution of genetic variation at the mitochondrial control region among beluga populations (Brown Gladden *et al*. 1997, O'Corry-Crowe *et al*. 1997) we found a relatively high degree of genetic structure. It is noteworthy that while many of the *P*-values were rendered insignificant after sequential Bonferroni corrections at the table-wide level, nine out of fifteen homogeneity tests yielded *P*-values of less than .05. This proportion is clearly much more than the expected proportion of approximate five percent of "random" *P*-values below .05, the reason for implementing the conservative Bonferroni procedure. The high proportion of low *P*-values is a clear indication of a biological cause of heterogeneity and not merely a stochastic phenomenon in the statistical analyses, suggesting a structured distribution of mitochondrial DNA variation within the Baffin Bay and West Greenland belugas.

Possible effects of a matrilineal pod structure

Maternally directed site-fidelity has previously been suggested as the cause of such a high degree of genetic structure by Brown Gladden *et al*. (1997) as well as O'Corry-Crowe *et al*.

(1997). Maternal structure within a single panmictic (random mating) population is indeed conceivable and has previously been observed among humpback whales and right whales (Palsbøll *et al*. 1995, Schaeff *et al*. 1993). However, if maternally directed site-fidelity to specific summer or winter grounds is the sole mechanism generating heterogeneity, would we then expect heterogeneity between samples collected in the same season and area across different years?

Our analyses detected a significant degree of heterogeneity between different samples collected during the autumn in Upernavik as well as a marginal *P*-value between Upernavik and Disko Bay samples (supposedly from the same breeding population). Earlier studies have only reported a single such incidence, namely by Brown Gladden *et al*. (1997). In their study only a single incidence of a significant degree of inter-annual heterogeneity was detected at Pangnirtung at southeastern Baffin Island. Hence, at a cursory first glance, it might seem as though inter-annual heterogeneity is a rare phenomenon. However, detecting significant levels of genetic heterogeneity among closely related samples depends critically upon sufficient sample sizes, which in turn determines the statistical power. On a closer inspection it appears that the *only* sample area where more than 15+ samples were collected and analysed in more than a single year by Brown Gladden *et al*. (1997) was indeed the Pangnirtung sample. In the present study, the Upernavik sample contained the most extensive inter-annual coverage. Hence, the rarity of inter-annual heterogeneity in the current and previous studies might equally well be due to insufficient sample coverage. In fact, in the few instances where sufficient samples have been available from multiple years, inter-annual heterogeneity has been detected, indicating it might be a common phenomenon, contrary to the first impression.

What might then be the cause of such inter-annual heterogeneity within one area? The samples collected in the Upernavik municipality were mainly from pods consisting of mature females accompanied by immature males and females, presumably the mature females and their offspring. Such social organization is not uncommon among odontocetes and has been shown to generate significant genetic heterogeneity among different pods in killer whales (Hoelzel *et al*. 1998, Hoelzel and Dover 1991) and sperm whales (Richard *et al*. 1996). Hence, it is quite conceivable that the inter-annual heterogeneity detected among belugas is due to analysing samples collected from different matrilineal pods. This possibility raises some issues in terms of how to interpret the genetic heterogeneity detected in this and previous genetic studies of belugas. If a matrilineal pod structure is contributing significantly to genetic heterogeneity among samples, how do we discriminate between different populations, seasonal aggregations and pods? Simply employing a significant degree of genetic heterogeneity between two sample partitions as a criteria to assign these to different management units might be misleading if such heterogeneity may be partly or solely due a matrilineal pod structure. The genetic analyses will thus need to take the co-existence of pods, seasonal aggregations and breeding populations into account, which so far has not been the case and is unlikely to be accommodated if samples are obtained from the harvest only.

Lack of population genetic signal

Irrespective of the degree of genetic differentiation among matrilineal pods, this would still be less than the degree of genetic divergence among breeding populations with low levels of gene flow. Consequently we expect the patterns of genetic divergence on a large geographical scale to be consistent; that is, geographically proximate samples are more closely related than they are with distant samples. Such an analysis must take (sometimes unknown) migratory patterns into account and should thus appropriately be confined to samples collected during the same season. The present study was conducted on a relatively limited geographical scale and we failed to detect any correlations between samples that were consistent with their spatial or temporal distribution, even when samples were partitioned according to what we know about the annual migration pattern. Some of the largest genetic distances were observed between proximate localities, such as Devon Island/Grise Fiord and Southeast Baffin Island as well as between Qaanaaq and mid-West

Table 5
The degree of genetic divergence (as K_{ST}) and the probability of homogeneity estimated between eastern Arctic Canada and West Greenland sampling areas

Canadian sampling areas			Greenland sampling areas			
	p	*n*	K_{ST}			
			Qaanaaq	Upernavik pre1992	Upernavik post1991	Disko/Maniitsoq and Sisimiut
Southeast Baffin Island	.00369 (.00100)	14	.085 (<.024)	0.0001 (<.41)	.036 (<.010)	.012 (<.10)
Devon Island & Grise Fiord	.00509 (.00213)	16	.043 (<.12)	.086 (<.013)	.0001 (<.80)	.015 (<.081)
Devon Island - Southeast Baffin Is.			.065 (<.028)			

Note. *p* denotes the estimated nucleotide diversity (Nei & Li 1979) with the standard error in parenthesis. *n* denotes the sample size. The numbers in parenthesis in the K_{ST} columns denote the probability of the observed value of K_{ST}. assuming the two samples were collected from the same population.

Greenland (Table 4). However the estimated genetic distance between both the Canadian sampling localities and mid-West Greenland are similar or several fold lower (Tables 4 and 5). The genetic distances between either of the Upernavik partitions and the remainder of the samples are highly inconsistent as well. This discrepancy between genetic and spatial/temporal divergence could be due to the fact that we were mainly estimating genetic divergence among pods, rather than populations/seasonal aggregations of belugas. However, it could also be due to an inadequate amount of data in relation to the degree of genetic divergence.

The study by Brown Gladden and co-workers (1997) was conducted on a much wider geographical scale, and provides an opportunity to inspect the distribution of genetic variation at higher regional levels. At least at higher levels of geographic separation (*e.g.* Baffin Bay and western Arctic Canada) we should expect to find that spatially proximate samples are genetically more similar than they are to spatially distant samples as migration patterns are unlikely to contribute to any confusion among such locations. The phylogenetic relationship estimated among the summer areas sampled from the mitochondrial control region sequences by Brown Gladden *et al*. (1997) revealed some surprising inconsistencies (Figure 3 in Brown Gladden *et al*. 1997). For instance, samples collected at summer grounds in the Beaufort Sea clustered together with samples from the Baffin Bay, and yet other Baffin Bay samples were grouped in a different clade, clustering with East Chukchi Sea summer ground samples. Affinities among such remote summer grounds demonstrate inconsistencies in the data that cannot be explained by migration patterns or pod-structure and are likely due to an insufficient phylogenetic signal. Insufficient phylogenetic signal could be due to multiple factors, such as too few samples, too few loci or base pairs analysed, or a combination of these.

Conclusion

Our study revealed a high degree of genetic structure among Baffin Bay belugas but little regarding the source of the genetic heterogeneity. In fact, the current study as well as earlier genetic analyses of belugas (Brown Gladden *et al*. 1997) strongly indicates that a simple straightforward interpretation of the current data is difficult. It is conceivable that a matrilineal pod structure adds significant levels of genetic heterogeneity and that the current sample sizes/loci contain insufficient phylogenetic signal for the purpose of defining meaningful management units. These apparent issues are by no means irresolvable but will have to be addressed by a dedicated sampling scheme and appropriate genetic analyses. For instance, the degree of kinship among samples is readily es-

timated from analysis of multiple (>20) microsatellite loci, and thus an insight into the contribution of a matrilineal pod structure can relatively easily be obtained. Similarly, a directed sampling scheme that ensures adequate samples from consecutive years from the same area and season within a number of locations would aid in the estimation of the contribution of interannual heterogeneity to the overall degree of genetic heterogeneity. Such a directed sampling scheme would probably require that samples were obtained from other sources in addition to the local harvest, for instance by biopsy sampling of free-ranging individuals.

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