Population structure and seasonal movements of narwhals, *Monodon monoceros*, determined from mtDNA analysis

PER JAKOB PALSBOELL*†, MADP PETER HEIDE-JØRGENSEN‡ & RONE DIETZ§

†Department of Population Biology, Zoological Institute, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, ‡Greenland Institute of Natural Resources, Tagensvej 135, 1, DK-2200 Copenhagen N and

§Department of Arctic Environment, National Environmental Research Institute, Tagensvej 135, 4, DK-2200 Copenhagen N, Denmark

We determined the nucleotide sequence of the first 287 base pairs in the mitochondrial control region from 74 narwhals, *Monodon monoceros*, collected in the North-west Atlantic. We detected four polymorphic sites that defined five haplotypes, two of which were found in single specimens. The same DNA sequence was characterized in an additional 353 specimens by digestion with two restriction endonucleases. In this manner each specimen could be assigned to one of the three most common haplotypes. The nucleotide diversity for the total sample (as well as the sequenced subset) was estimated as 0.0017 and pairwise genetic distances between haplotypes ranged from 0.0035–0.0070. The low nucleotide diversity and the low average pairwise genetic distance between haplotypes suggest a recent expansion in abundance from a small founding population. Despite the low degree of variation, frequencies of the common haplotypes differed markedly between areas. The results indicate isolation, even between geographically close areas, as well as fidelity to specific summer and autumn feeding grounds. Heterogeneity within a presumed single breeding ground suggests mixing of pods with different haplotypic composition.

Keywords: Cetaceae, D-loop, evolution, low nucleotide diversity, Odontocetæ, population genetics.

Introduction

The narwhal, *Monodon monoceros*, summers in open water areas of the High Arctic but spends the winter in close association with pack ice at lower latitudes (Hay & Mansfield, 1989). Although narwhals appear to be well adapted to the High Arctic environment, the regular occurrence of 'sassa', the entrapment by ice and subsequent extinction of entire pods, demonstrates the unpredictability of this habitat (Siegestad & Heide-Jørgensen, 1994).

Narwhals are usually encountered in pods composed of either mature females accompanied by calves and immature males or solely of mature males. The female pods are believed to be matrifocal and all mature females appear to reproduce. Mating is believed to occur once a year, in the spring or early summer on the wintering grounds (Hay & Mansfield, 1989).

The current estimate of abundance for the combined Canadian and Greenland parts of Baffin Bay and adjacent waters is between 27 600 and 42 500 (IWC, 1992). No population estimates exist for eastern Greenland, but the relatively low number of catches and sightings in the area suggests a much smaller population relative to that of Baffin Bay (Dietz *et al.*, 1994).

*Correspondence: Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717, U.S.A.
E-mail: ppalsbol@newt.bio.uci.edu

Our present limited knowledge concerning patterns of annual migration and population structure in this species comes mainly from hunting records. The seasonal timing of catches indicates that West Greenland narwhals spend the summer in the high-latitude Avanersuaq district, then migrate southwards during the autumn (where they are hunted in the districts of Upernavik and Uummannaq) to winter in Disko Bay (Fig. 1). However, narwhals have also been observed offshore in the heavy pack ice in the Davis Strait as well as in central Baffin Bay (Koski & Davis, 1994). In East Greenland narwhals are observed along most of the outer coast north of 64°N (Dietz *et al.*, 1994). The paucity in sightings of narwhals as well as the lack of

recordings of trade with tusks, meat or mattak (skin) south of Sisimiut (66°N) suggests that no migration occurs between East and West Greenland (IWC, 1992; Reeves & Heide-Jørgensen, 1994). Recently, satellite-linked radio transmitters have been employed to map the movements of narwhals in West Greenland. During the summers of 1993 and 1994 nine narwhals captured in Melville Bay were instrumented with satellite transmitters (Dietz & Heide-Jørgensen, 1995). Two of the transmitters were monitored for 100 days. In both instances the instrumented narwhals migrated south along the western coast of Greenland, following the 1000 metre depth curve and wintered in the pack ice midway between Canada and Greenland. Thus the

Fig. 1 Map of narwhal sampling localities. Localities in italics are where samples were obtained from sassaqs (see text).

migration pattern observed from the instrumented narwhals confirmed the impression from the hunting records, as well as providing additional information from areas where no hunting takes place.

Here we present the results of a population genetic study based upon analysis of the first 287 nucleotides in the mitochondrial (mt) control region. We analysed 427 narwhal samples obtained from summer, autumn and winter grounds in eastern and western Greenland as well as eastern Canada. The mt genome has previously been successfully applied as a genetic marker in studies of population structure in cetacean populations (Schaeff *et al.*, 1991; Baker *et al.*, 1993; Palsbøll *et al.*, 1995). The maternal mode of inheritance and lack of recombination in this genome is an additional advantage for population studies of species (as is the case for many cetaceans) where site fidelity and/or pod structure are maternally directed.

Methods

Sample collection and storage

Samples of skin and muscle were collected by biologists or Inuit hunters during flensing of narwhals between 1984 and 1994, at the locations shown in Fig. 1. Samples were stored at -28°C or in saturated sodium chloride with 20 per cent DMSO (dimethylsulphoxide) until analysis (Amos & Hoelzel, 1991). From 1990 onwards, tissue samples were also collected from lower jaws supplied by the Inuit hunters. The jaws were frozen and mailed to the Greenland Institute of Natural Resources.

On two occasions (at Savissivik in February, 1994, and Kitsissuarsuit in March, 1994 — see Fig. 1), samples were collected from entire pods (100–200 individuals) which died in ice entrapments (Heide-Jørgensen, unpublished information). All other samples were collected from single narwhals taken from small ($n < 20$) pods.

DNA extraction and analysis of the mt control region

DNA was extracted using standard procedures by cell lysis with SDS (sodium dodecyl sulphate) followed by digestion with Proteinase K at 65°C . Degraded proteins were removed by several phenol/chloroform extractions and finally the nucleic acids were precipitated with ethanol (Maniatis *et al.*, 1982). Single-stranded mtDNA for dideoxy sequencing (Sanger, 1981) was obtained by symmetric amplification and subsequent asymmetric amplification

with AmpliTaq DNA polymerase (Gyllenstein & Erlich, 1988; Saiki *et al.*, 1988). Symmetric amplifications of the whole mt control region were conducted with the primers MT3R and MT4F annealing to sequences flanking the mitochondrial control region in cetaceans (Arnason *et al.*, 1993). Amplifications were performed in $20\text{ }\mu\text{L}$ under the following conditions: 67 mM TrisCl, pH 8.8, 2 mM MgCl_2 , 16.6 mM $(\text{NH}_4)_2\text{SO}_4$, 10 mM β -mercaptoethanol, 0.20 μM per nucleotide, 1 μM for each primer, 0.4 units AmpliTaq DNA polymerase and $\approx 10\text{ ng}$ extracted genomic DNA. The temperature profile was: 1 min at 94°C , 1 min at 57°C and finally 3 min 30 s at 72°C for 28 cycles. Asymmetric amplifications were conducted with the primers MT4F and Mn312R (Arnason *et al.*, 1993; Palsbøll *et al.*, 1995). Amplifications were performed under similar conditions as the symmetric amplifications except that the reaction volume was $50\text{ }\mu\text{L}$, the annealing temperature 55°C and the extension time 1 min 30 s for 30 cycles. All extractions and amplifications included blanks as controls for possible contamination. Sequencing was performed according to the manufacturer's instructions (Sequenase Ver. 2.0, US Biochemicals). The primers were MT4F, Mn312R and Mn152R (Palsbøll *et al.*, 1995).

Restriction endonuclease analysis

Digestions of double-stranded PCR products with the endonucleases *Mnl*I and *Bgl*II were performed in $20\text{ }\mu\text{L}$ volume under the conditions recommended by the manufacturer (New England Bio Labs). The restriction fragments were visualized by electrophoresis through 2 per cent NuSieveTM and staining with ethidium bromide.

Sex determination

The sex of each sample was determined using a PCR-based method (Bérubé & Palsbøll, 1996).

Data analysis

The sample localities were divided into three main areas: eastern Greenland, western Greenland and northern Baffin Bay. Northern Baffin Bay includes eastern Canada and the Aversersuaq district. Although a total of 29 samples was collected in eastern Canada, only one locality (Pond Inlet) had a reasonable sample size of 19 specimens. For this reason only Pond Inlet samples were used in the homogeneity tests as representing eastern Canada. Western Greenland includes the localities Balgøni

	1	11	21	31	41	51
<i>D. delphis</i>	AAAAA-GCTT	ATTGTACAAT	TACCACAACA	TCACAGTACT	ACGTCAGTAT	TAAAAGTAAT
<i>M. monoceros</i>	..G..G.T..	T.-	..T..A.C..	.T.....G..		A--.C
	61	71	81	91	101	111
<i>D. delphis</i>	TTGTTTTTAAA	AACATTTTAC	TGTACATATT	ACATACACAT	ACACATGTAC	ATGCTAATAT
<i>M. monoceros</i>	C.A...C..T	-.....	GC....	G.....T.GC	.T...CAC..	G.CA...ATC
	121	131	141	151	161	171
<i>D. delphis</i>	TTAGTCTCTC	CTTGTAATA	TTCATATATT	CATGCTATGT	ATTATTGTGC	ATTCATTTAT
<i>M. monoceros</i>	T..	...A.....	.C.....	...A.....		
	181	191	201	211	221	231
<i>D. delphis</i>	TTTCATACG	ATAAGTTAAA	GCTCGTATTA	ATTATTATTA	ATTTTACATA	TTACATAATA
<i>M. monoceros</i>		G.C.....		.A.....	A.	.C.....
	241	251	261	271	281	
<i>D. delphis</i>	TGCATCCTCT	TACATATTAT	ATATCCCCTT	CAATTTTCATT	TCCATTATA	
<i>M. monoceros</i>	G....		..G.....A.	TC....T...		

Fig. 2 Alignment of the sequenced 287 nucleotides in *Monodon monoceros* with *Delphinus delphis*.

Øer (in the Melville Bay), Upernavik district, Uummannaq district and Disko Bay.

The nucleotide diversity was estimated as defined by Nei (1987; p. 256). The degree of genetic isolation between partitions was estimated as H_{ST} (Hudson *et al.*, 1992) and tested for statistical significance by comparison with 1000 Monte Carlo simulations (Hudson *et al.*, 1992). Rare haplotypes were not lumped together (Roff & Bentzen, 1992; Hudson, 1992). Samples were considered heterogeneous if a similar or more extreme value of H_{ST} was obtained in less than 5 per cent of the Monte Carlo simulations. The homogeneity tests were conducted in a hierarchical manner: males vs. females within each sampling locality; year to year; and neighbouring sampling localities. Partitions showing no significant degree of heterogeneity were pooled in subsequent tests.

Results

Sequence analysis

The complete nucleotide sequence of the first 287 base pairs of the mt control region was determined for 74 specimens. The consensus sequence, aligned with the homologous sequence in the common dolphin, *Delphinus delphis* (GeneBank accession no. M199017 (Rosel *et al.*, 1994)), is shown in Fig. 2.

		1	2	2
	5	1	3	6
	8	7	2	5
Mm001	—	A	C	C
Mm002	.	.	.	T ¹
Mm003	A	.	.	T ¹
Mm004	.	.	T	.
Mm005	.	G ²	.	.

Fig. 3 Polymorphic sites in the observed haplotypes. Vertical numbers indicate the position relative to the consensus sequence in Fig. 2. A dash (-) symbolizes a deletion/insertion introduced to optimize alignment.

¹Substitution creates a recognition site for the restriction endonuclease *MnI*. ²Substitution creates a recognition site for the restriction endonuclease *BglII*.

Only four polymorphic sites were detected, which defined five haplotypes (Fig. 3.). Apart from one insertion all polymorphisms were transitions. Of the 74 sequenced specimens all but seven had a sequence of either haplotype Mm001 or haplotype Mm002.

Restriction analysis

The 287 base-pair sequence was amplified in an additional 353 specimens and the product digested

Table 1 The number of narwhal samples of each haplotype and gender from each sampling area

	Mm001	Mm002	Mm003	Mm004	Mm005
<i>Eastern Canada</i>					
Females	13	4	—	1	1
Males	9	—	—	—	—
<i>Avanersuaq district</i>					
Females	49	17	1	—	3
Males	71	26	—	—	—
<i>Melville Bay</i>					
Females	6	3	—	—	—
Males	3	8	—	—	—
<i>Upernavik district</i>					
Females	1	1	—	—	—
Males	5	4	—	—	—
<i>Disko Bay</i>					
Females	7	7	—	—	—
Males	1	8	—	—	—
Unknown gender	5	—	—	—	—
<i>Disko Bay 1994</i>					
<i>sassat</i>					
Females	18	5	—	—	—
Males	21	6	—	—	1
<i>Uummannaq district</i>					
Females	21	2	—	—	—
Males	60	5	—	—	—
<i>Eastern Greenland</i>					
Females	—	17	—	—	—
Males	—	11	—	—	—

with the restriction endonucleases *Mnl*I and *Bgl*II. In this manner the mt control region of each specimen could be identified as either Mm002 or Mm003 (cut by *Mnl*I only), Mm005 (cut by *Bgl*II) or one of either Mm001 or Mm004 (no restriction sites for either *Mnl*I or *Bgl*II) (Fig. 3).

Sex determination

Sex was determined for 416 specimens (11 samples resisted proper amplification). Sex and mt control region haplotypes are summarized by year for the sample areas across years in Table 1.

Nucleotide and haplotype diversity

The estimated nucleotide diversity for the 74 sequenced specimens was 0.0017. The genetic distance between haplotypes varied from 0.0035–0.0070. The estimated nucleotide diversity for all 427

specimens was also 0.0017 ignoring the haplotypes Mm003 and Mm004.

Homogeneity tests

No heterogeneity was detected between sexes in any area. Within the northern Baffin Bay (including the sassat samples from Savissivik in February, 1994) and eastern Greenland (monomorphic for haplotype Mm002) no heterogeneity was detected between years or sampling localities. In western Greenland, two instances of significant levels of heterogeneity were found: the samples from Uummannaq district differed in haplotype frequencies from the rest of the western Greenland sampling localities; and the samples originating from the sassat at Kitsissuarsuit in May 1994 differed from the Uummannaq samples as well as from the rest of the western Greenland samples, between which no significant levels of heterogeneity were detected. In summary, the homogeneity tests identified five 'entities': (i) northern Baffin Bay, (ii) eastern Greenland, (iii) Uummannaq district, (iv) the 1994 sassat at Kitsissuarsuit and (v) the remaining western Greenland localities (Melville Bay, Upernavik district & Disko Bay, excepting the 1994 sassat samples). The H_{ST} values for these partitionings (along with the levels of significance) are listed in Table 2.

Discussion

Low levels of nucleotide and haplotype diversity

The current population of narwhals in the Baffin Bay region is relatively large when compared to many other cetacean species. Thus, it is surprising that the estimated nucleotide diversity is 0.0017, which is one of the lowest values reported to date for any cetacean species (Table 3). Similar values have so far been observed only in cetacean populations which either number few animals (in the range of 300–500 individuals), and/or are assumed to have been founded recently by a few individuals (Table 3). The sequenced samples included specimens collected over a wide geographical range and time span (see Table 2). Hence the estimate of the nucleotide diversity is likely to be representative for the overall North-west Atlantic distribution of this species *per se*. Our findings resemble the results reported by Hoelzel *et al.* (1993) in a study of the northern elephant seal, *Mirounga angustirostris*, in which they reported an equally low nucleotide and haplotype diversity (two haplotypes with a genetic distance of 0.01). Today, northern elephant seals are

Table 2 Results from homogeneity tests between narwhal samples (H_{ST} values and level of significance)

	Eastern Canada & Avanersuaq district	Disko Bay	Disko Bay 1994 sassat	Eastern Greenland	Melville Bay & Upernavik district	Uummannaq district
Eastern Canada & Avanersuaq district	—	0.0166*	NS	0.24***	0.040***	0.038***
Disko Bay		—	0.046*	0.35***	NS	0.18***
Disko Bay 1994 sassat			—	0.53***	0.094**	0.033*
Eastern Greenland				—	0.27***	0.73***
Melville Bay & Upernavik district					—	0.25***
Uummannaq district						—

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$; NS, nonsignificant.

Table 3 Estimates of nucleotide diversity in other cetacean populations with low levels of genetic variation

Species	Data	Nucleotide diversity	Source
† <i>Stenella longirostris</i>	RFLP of total mt genome		
Timor Sea	$n = 11$	0.003	Dizon <i>et al.</i> (1991)
Eastern Pacific	$n = 79$	0.012	Dizon <i>et al.</i> (1991)
<i>Orcinus orca</i>	mt control region sequence		
North-eastern Pacific	$n = 8$	0.002	Hoelzel & Dover (1991)
<i>Eubalaena glacialis</i> ,	RFLP of total mt genome		
North Atlantic	$n = 10$	0.0026	C. Schaeff, pers. comm.
† <i>Megaptera novaeangliae</i>	mt control region sequence		
Hawaii	$n = 7$	0.00	Baker <i>et al.</i> (1993)
West Greenland	$n = 44$	0.026	Palsbøll <i>et al.</i> (1995)

†For species where two estimates are given, the second estimate is an example of more commonly observed levels of nucleotide diversity within that particular species.

abundant ($\approx 120\,000$ individuals), but the population underwent a severe bottleneck (< 30 individuals) only a century ago because of intense hunting pressure.

Nothing is known concerning the past abundance of narwhals, but the data presented here strongly suggest that the current population was founded from only a few individuals relatively recently. The term 'recently' should be viewed in the proper context, which (taking the substitution rate into account) could be several tens of thousands years ago.

Such expansion could have been facilitated by the retreat of the ice after a glaciation and the consequent greater availability of habitat suitable for narwhals.

Another explanation which has been suggested to account for the low mt diversity, is the apparent matrifocal pod structure. Behavioural as well as genetic studies have demonstrated a matrifocal pod structure in several species of odontocetes (Amos *et al.*, 1991, 1993; Duffield & Wells, 1991). Hence, if a population is subdivided into several maternal pods, genetic drift will cause a reduction of the number of maternal lineages within each pod. The result will be a reduction (relative to a nonmatrifocal, panmictic population) in the nucleotide diversity (for mtDNA) within each pod. However, because each pod constitutes an independent maternal lineage it will diverge from maternal lineages (other pods) with time. Thus the effects of a matrifocal pod structure will not affect the nucleotide diversity at the population

level, because different pods randomly become fixed for different haplotypes (especially in large populations such as that of the Baffin Bay region). Consequently, the matrifocal pod structure cannot explain the observed low nucleotide diversity across the entire geographical range included in this study. The cause proposed here, a rapid and recent expansion from a small founding population, seems the most plausible explanation for the unusually low level of diversity observed.

Heterogeneous geographical distribution of mt haplotypes

Despite the low overall variation in the mt control region in the narwhal we still observed marked and significant differences in haplotype frequencies between sampling areas (Table 1).

Eastern Greenland

The fact that the eastern Greenland samples were monomorphic supports the hypothesis of little (or no) gene flow between eastern and western Greenland (IWC, 1992; Reeves & Heide-Jørgensen, 1994).

Summer grounds in the Baffin Bay region

In the Baffin Bay region we analysed samples from summering grounds in eastern Canada, Avanersuaq district and Melville Bay. No significant levels of heterogeneity were found within the northern Baffin Bay (eastern Canada and Avanersuaq district) indicating some gene flow between summer grounds within this area. Furthermore, within the Avanersuaq district, where samples were available from several years, haplotype frequencies appeared to be similar from year to year. Although the Melville Bay area is separated by only a few hundred kilometres from the Avanersuaq summer grounds, we found a significant level of heterogeneity between the northern Baffin Bay summer grounds and those of Melville Bay. Hence little gene flow is occurring between the western Greenland summer areas and northern Baffin Bay. However, it should be kept in mind that the sample size from Melville Bay ($n = 20$ over two years) is quite low.

Autumn and winter localities in the Baffin Bay region

From the hunting records it is believed that narwhals summer in the northern Baffin Bay; migrate southward during the autumn; pass through

the districts of Upernavik and Uummannaq, where they are hunted; and winter in Disko Bay and mid-Baffin Bay. The pairwise comparisons between sampling localities within the western Greenland area confirm the impression of an autumn southward migration along the coast to Disko Bay, but starting in the Melville Bay if we disregard the samples collected in Uummannaq district and from the sassat in the northern Disko Bay (Kitsissuarsuit) in 1994. The somewhat surprising finding that the narwhals taken in Uummannaq were heterogeneous when compared with those collected in Melville Bay and the Upernavik district, has been supported by recent satellite monitored trackings of narwhals (Dietz & Heide-Jørgensen, 1995). Individual narwhals tracked by satellite in Melville Bay showed only limited movements within the summer area, hence confirming the findings from this study of little gene flow between northern Baffin Bay and Melville Bay. In the autumn the instrumented narwhals migrated southward following the 1000 metre depth curve. At the time when narwhals were observed and hunted in the Uummannaq district, the tagged narwhals had already arrived in their winter grounds, in the pack ice 200 km west of Disko Bay.

In Disko Bay sample sizes were generally small, except for the 58 samples collected from the sassat at Kitsissuarsuit in 1994. The sassat sample has haplotype frequencies which differ from all other partitionings apart from the northern Baffin Bay samples. The relatively rare haplotype Mm005 was found only in the northern Baffin Bay and the Disko Bay 1994 sassat samples. This could indicate that narwhal pods from different summer areas (in this case western Greenland and northern Baffin Bay) share the same breeding ground. That not all pods in the northern Baffin Bay area migrate southward and winter in the central Baffin and/or Disko Bays was demonstrated by the samples collected from the sassat at Savissivik (Avanersuaq district) in February 1994. This sample had haplotype frequencies similar to the other localities within northern Baffin Bay.

In conclusion, the overall picture emerging from our analyses is consistent with the satellite trackings, and suggests an annual fidelity to relatively discrete summer and autumn feeding grounds. Our data suggest that pods of different genetic composition (summer feeding grounds?) utilize the same winter ground (here represented by Disko Bay). Maternally directed philopatry to separate summer feeding grounds (within a single breeding population) has been observed to affect the distribution of mtDNA haplotypes in other cetacean species (ex Palsbøll *et*

al., 1995). Amos *et al.* (1993) have suggested (for pilot whales, *Globicephala melaena*) that pods may merge briefly during the mating season to facilitate outbreeding. The results presented here suggest that such a breeding system may also exist for narwhals.

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