Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the western Nearctic revealed by mitochondrial DNA

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Abstract

The recent evolutionary history, population structure and movement patterns of beluga whales in the western Nearctic were inferred from an analysis of mitochondrial DNA control region sequence variation of 324 whales from 32 locations representing five summer concentration areas in Alaska and north-west Canada. Phylogenetic relationships among haplotypes were inferred from parsimonious networks, and genetic subdivision was examined using haplotypic frequency-based indices and an analysis of variance method modified for use with interhaplotypic distance data. MtDNA relationships were characterized by a series of star-like phylogenies which, when viewed in conjunction with information on haplotype frequency and distribution, suggested a rapid radiation of beluga whales into the western Nearctic following the Pleistocene, and an early divergence of the Beaufort Sea from the Chukchi and Bering Seas subpopulations. Overall nucleotide diversity was low (0.51%) yet all major summering concentrations were significantly differentiated ($\Phi_{ST} = 0.33$) from one another. Stratification of samples by gender and age from the three northernmost subpopulations suggested that female cohorts from neighbouring subpopulations were more differentiated than males. Further stratification of adult animals by age revealed that older adults were substantially less subdivided among locations than younger adults, particularly for males, suggesting that dispersal, although limited, is biased toward older adult males. Overall, the patterns of mtDNA variation in beluga whales indicated that the summering concentrations are demographically, if not phyletically distinct. Population structure appears to be maintained primarily by natal homing behaviour, while asymmetries in dispersal may be associated with the type of mating system.

Keywords: dispersal, beluga whale, mitochondrial DNA, population structure, mating system

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Introduction

Knowledge of the population structure and dispersal patterns of cetacean species is an important prerequisite to their effective management, yet information on such aspects of the lives of whales and dolphins is difficult to obtain because of their relative inaccessibility. The challenges are particularly acute in wide-ranging species that exhibit complex migration patterns. However, with

the development of molecular genetic techniques, it is now possible to assess levels of genetic relatedness among populations and other natural groupings and to gain insights into patterns of dispersal, mating systems, and the role of kinship in group structure (Amos *et al*. 1993; Avise 1994; Baker *et al*. 1994). Using sequence variation within the mitochondrial genome, we examined population structure of the beluga whale *Delphinapterus leucas* in the western Nearctic. More generally, we explored the utility of this molecular marker in elucidating patterns of dispersal and gene flow among natural populations.

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The beluga whale is a highly social, vagile species that exhibits great variation in movement patterns and group structure. Over much of its range, this medium-size toothed whale migrates hundreds, or even thousands, of kilometres each year between wintering grounds in the pack ice and summering grounds in warm coastal and adjacent offshore waters of the arctic and subarctic (Kleinenberg *et al*. 1964; Frost & Lowry 1990; Richard *et al*. 1990). They tend to follow established migration routes and occupy geographically distinct traditional summering areas, often in large numbers, where they molt, feed, and rear their young (Seaman *et al*. 1982; St. Aubin *et al*. 1990; Caron & Smith 1990; Smith *et al*. 1992, 1994). Occasional resightings of tagged and naturally marked individuals at a number of these sites indicate that beluga whales may return to the same summering area for more than 1 year (Sergeant 1973; Caron & Smith 1990).

Apart from their well-documented migratory movements, disjunct summer distribution and the limited evidence of philopatry, little is known about the population structure of beluga whales. For example, although no direct evidence exists, some summer concentrations are believed to share the same wintering grounds (Smith & Hammill 1986; Frost & Lowry 1990; Richard 1991, 1993). As breeding takes place primarily between the months of April and July (Braham 1984; Burns & Seaman 1988; Brodie 1989; Heide-Jørgensen & Teilmann 1994), interbreeding, as well as individual exchange, among geographical summer concentrations may occur on the common wintering ground or during spring migration. Furthermore, considering the vast distances over which beluga can range,

there may be other opportunities for dispersal among summer concentrations, or in some cases even among wintering grounds. Our understanding of which individuals disperse is even more limited, nor is it clear when and why they disperse.

Beluga whales have long been an important subsistence resource for many coastal aboriginal peoples (McGhee 1974; Lowry *et al*. 1989; Heide-Jørgensen 1994; Savelle 1995). Their persistence in returning to nearshore waters, however, enabled past commercial operations to hunt some populations to the point of economic extinction and has made this species particularly vulnerable to other human-related pressures (e.g. disturbance, habitat loss and pollution; for review see O'Corry-Crowe & Lowry, 1997). Of immediate concern is the definition and identification of separate, biologically relevant management stocks and the preservation of genetic diversity. In general, management stocks of beluga whales, world-wide, are based on nonuniform patterns of distribution during the summer months (Smith & Hammill 1986; Frost & Lowry 1990). Differences in body size, contaminant levels, and population trends among these summer concentrations have also been interpreted as indicative of stock discreteness (Sergeant & Brodie 1969; Mitchell & Reeves 1981; Muir *et al*. 1990; Wagemann *et al*. 1990; Doidge 1990; Stewart 1994). These approaches to stock identification, however, have limitations due to incomplete knowledge on year-round distribution, movement patterns, and breeding behaviour. Specifically, they provide little or no information on rates and form of individual and genetic exchange, and although phenotypic differences are highly
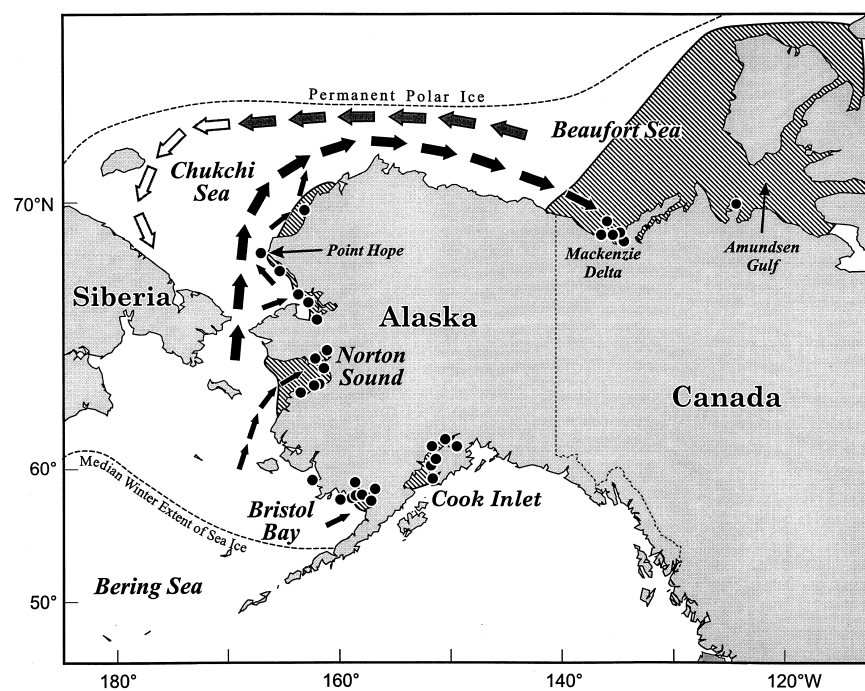
Fig. 1 The five major summering areas (hatched pattern) of beluga whales in Alaska and north-west Canada. From north to south: eastern Beaufort Sea, eastern Chukchi Sea, Norton Sound, Bristol Bay and Cook Inlet. Sampling locations are indicated by small dark circles. Black arrows represent spring migration routes, grey arrows represent known autumn routes, and white arrows represent hypothesized autumn routes

Table 1 Locations from which beluga whales were sampled, with respective sample sizes, haplotype and nucleotide diversities

Location	No. of sampling sites	Sample size	Haplotype diversity (H)	Nucleotide diversity (%)
Cook Inlet	6	37	0.524	0.23
Bristol Bay	8	24	0.163	0.15
Norton Sound	6	66	0.492	0.19
Eastern Chukchi Sea	5	103	0.736	0.57
Eastern Beaufort Sea	7	94	0.702	0.38
Mackenzie Delta & Amundsen Gulf	6	69	0.687	0.35
Point Hope	1	25	0.753	0.46
All locations	32	324	0.844	0.51

suggestive, they may not provide evidence of evolutionary uniqueness (*sensu* Ryder 1986; Waples 1991; Dizon *et al.* 1992; Moritz 1994).

Five separate management stocks have been proposed for waters off Alaska and north-west Canada (Fig. 1; Frost & Lowry 1990). A small, geographically isolated stock inhabits Cook Inlet. The four remaining stocks: Bristol Bay, Norton Sound, the eastern Chukchi Sea and the eastern Beaufort Sea represent geographically distinct summering concentrations (Fig. 1). No obvious geographical barriers separate these four stocks. All may share a common wintering ground in the pack ice of the central Bering Sea and are referred to collectively as the Bering Sea population (Burns & Seaman 1986; Frost & Lowry 1990). Under this scenario at least three hypotheses of stock discreteness exist: (i) there is regular exchange of individuals between summering concentrations; (ii) interbreeding occurs between these concentrations on wintering grounds, or possibly during migration, but individuals remain philopatric to their summering grounds; or (iii) there is no individual exchange or breeding between summer concentrations, either on the summering or wintering grounds. Thus, depending on the strategy pursued, population genetic structure can range from one of potentially substantial genetic exchange among summering groups to one where these groups constitute discrete evolutionary units that appear to winter in a common area but remain nonetheless genetically isolated. The reality, however, is that little is known about the winter distribution of any of the proposed stocks, including Cook Inlet, and the interactions between summering concentrations in winter and spring may be quite complex.

In this paper we examine the population genetic structure and movement patterns of beluga whales in Alaska and north-west Canada using nucleotide sequence variation within the mitochondrial genome's control region. Because of its predominantly maternal mode of inheritance (Hutchison *et al.* 1974; Avise 1986), the effective number of mtDNA genomes is one quarter that of nuclear genes, leading to a more rapid rate of genetic differentiation through random drift (Birky *et al.* 1983). Genetic divergence is also enhanced by the higher rate of sequence evolution (in vertebrates at least), particularly within the hypervariable section of the control region, relative to nuclear DNA coding regions (Brown *et al.* 1979; Brown 1983; Moritz *et al.* 1987). These properties also facilitate the reconstruction of phylogenetic relationships among mtDNA haplotypes, which can be compared with their present day geographical distribution to assess the processes governing population genetic structure (Avise *et al.* 1987). The almost exclusive maternal mode of inheritance for this genome however, is generally seen as limiting its value in studies on mating systems and behavioural ecological differences between the sexes. However, here we demonstrate that mtDNA sequence data from a substantial number of individuals, when used in conjunction with ancillary biological data, can indeed be informative about patterns of dispersal and gene flow.

Materials and methods

Sample collection and DNA extraction

Whales were sampled from 32 separate locations, representing five summering areas and one spring migration route, in the waters adjoining Alaska and north-west Canada over an 18-year period from 1977 to 1995 (Fig. 1, Table 1). Specimens sampled were either from individuals taken during aboriginal hunts or from beachcast animals. Two tissue types were used: liver stored at –80 °C and skin preserved in 20% (v/v) dimethyl sulphoxide (DMSO) saturated with sodium chloride (Amos & Hoelzel 1991). Total cellular DNA was isolated using standard phenol–chloroform and ethanol precipitation procedures outlined in Sambrook *et al.* (1989). The concentration and quality of resultant DNA was estimated by spectrophotometry and visualized on 1% agarose gels.

Amplification of mtDNA

Target DNA was amplified using the PCR (Saiki *et al.* 1988) in 100-µL reactions containing 10–100 ng genomic

DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl$_2$, 0.01% gelatin, 150 μM of each dNTP, 0.3 μM of each primer, and 2.5 units of *Taq* DNA polymerase. The profile for the Perkin Elmer (PE) thermocycler consisted of 1.5 min denaturation at 94 °C, 2 min annealing at 48 °C, and 3 min extension at 72 °C for 35 cycles followed by a final extension period of 5 min at 72 °C. Part of the threo-nine tRNA gene, the complete proline tRNA gene, and adjacent 5′ end of the control region of the mitochondrial genome were amplified with the following primers [numbers refer to the position on the human mtDNA corresponding to the primer's 3′ end (Anderson *et al.* 1981)]: L15926 (5′-ACACCAGTCTTGTAAACC-3′) modi-fied from Kocher *et al.* (1989) and H00034 (5′-TACCAAATG-TATGAAACCTCAG-3′) from Rosel *et al.* (1994). PCR prod-ucts were purified through Microcon® 100 (Amicon) microconcentrators and stored for up to six months at 4 °C.

Sequencing of mtDNA

Both strands of the amplified product were sequenced by dideoxy sequencing (Sanger *et al.* 1977) using Applied Biosystems' (ABI) four-dye fluorescent technology. Sequencing reactions were carried out in 20-μL reactions containing 20–200 ng of cleaned PCR product, 0.15 μM primer, and 9.5 μL ABI PRISM™ Ready Reaction DyeDeoxy™ terminator premix. A modified form of the AmpliTaq® polymerase, FS, was also used. Cycle sequencing was performed in a PE 9600 thermocycler pre-heated at 96 °C, followed by 25 cycles of 10 s denaturing at 96 °C, 5 s annealing at 50 °C, and 4 min extension at 60 °C. The light strand was sequenced with the L15926 primer; the heavy strand was sequenced with an internal primer, H16498 (5′-CCTGAAGTAAGAACCAGATG-3′, Rosel *et al.* 1994). Unincorporated dye-labelled terminators were removed from sequences by centrifugation through Centri-Sep™ spin columns, or in the case of the AmpliTaq® FS kit, by ethanol precipitation. Sequences were analysed on an ABI 373A Automated Sequencer, and data were edited and aligned with the SeqEd™ multiple sequence editor program (ABI 1992).

Gender identification

The sex of each whale was determined by (i) physical examination or (ii) molecular genetic analysis. The two methods used in the latter were both based on the detec-tion of a marker on the Y chromosome using the PCR. The first involved the coamplification of a sequence from the X- and Y-chromosome-linked zinc-finger protein genes (ZFX and ZFY) with a sequence from the Y-linked SRY gene (S. R. Fain, J. P. LeMay, personal communica-tion). Amplification of the zinc-finger gene homologues acted as an internal control for amplification success while the SRY sequence was the male determinator. The second method involved the amplification of a somewhat larger sequence of ZFX/ZFY followed by a restriction digest with *Taq*I endonuclease to reveal sex-specific differences due to sequence divergence among the ZFX and ZFY genes (Palsbøll *et al.* 1992).

Age and body size

When possible, information on standard length (to the nearest cm) was collected. Ages were determined based on the number of dentinal layers, termed growth layer groups (GLGs), counted in a thin longitudinal section taken from the middle of a mandibular tooth (Sergeant 1959; Brodie 1969). The removal, cutting and reading of teeth are described by Burns & Seaman (1986). Two GLGs were assumed to be laid down each year (Brodie 1982; Goren *et al.* 1987).

Analysis of mtDNA variation

The amount and nature of DNA polymorphism within the control region was assessed by estimating both haplotypic (Nei & Tajima 1981) and nucleotide (Nei 1987) diversity. To test for selective neutrality in the DNA region under examination, we used Tajima's (1989) *D*- statistic.

A number of approaches were used to reconstruct the phylogenetic relationships among the mtDNA sequences. Inference of evolutionary relationships were made using maximum parsimony analysis with PAUP 3.1 (Swofford 1993). The heuristic search algorithm was used and all minimum trees were saved. Maximum parsimony analy-sis of intraspecific mtDNA data, however, typically results in large numbers of equally parsimonious trees due to homoplasy (parallel mutations and reversals) and low numbers of informative sites. Consensus trees are highly polytomous and it is often difficult to distinguish true polytomies from ambiguities.

Because of this problem, phylogenetic relationships among the nucleotide sequences were also inferred from a minimum spanning network of the unique haplotypes (Excoffier *et al.* 1992; Excoffier & Smouse 1994; Bandelt *et al.* 1995). With the aid of the MINSPNET (Minimum Spanning Network, Excoffier & Smouse 1994) and NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System, Rohlf 1990) computer programs, haplotypes were connect-ed by a series of mutational events to all other haplotypes through a set of equally parsimonious pathways. Intermediate consensus haplotypes were reconstructed to obtain most parsimonious connections among haplotypes. In these networks, haplotypes serve both as nodes and branch tips and all character conflicts are included in the form of reticulations. Optimal trees were chosen from the set of alternative minimum spanning trees (MSTs) by

incorporating information on haplotype frequency and geographical location (Excoffier *et al*. 1992; Crandall & Templeton 1993; Excoffier & Smouse 1994). We searched for these 'most probable' evolutionary pathways using the criteria of Excoffier & Smouse (1994): (i) because there is a higher probability that a new haplotype will derive from a common haplotype (Excoffier & Langaney 1989; Crandall & Templeton 1993; Bandelt *et al*. 1995), a link between a rare haplotype and a common haplotype is more likely than a link among two rare haplotypes; (ii) in the absence of extensive gene flow, a recent mutation is more likely to be found in its original population than in some distant population (Slatkin & Maddison 1989; Crandall & Templeton 1993).

Two main approaches were used to measure the degree of genetic differentiation among geographical, age and sex strata. The first involved an analysis of variance method modified for use with molecular data (AMOVA, Excoffier *et al*. 1992). This approach incorporates information on nucleotide differences among haplotypes as well as haplotype frequency into a standard ANOVA format. The significance of variance components and *F*-statistic analogues, designated Φ-statistics, were tested by multiple permutation (1000×) of the original data set. The model we used for estimating the evolutionary distances between pairs of sequences was that of Tamura & Nei (1993). Pairwise distances were calculated using the MEGA version 1.0 computer program (Kumar *et al*. 1993).

The second approach utilized a number of test statistics that are based solely on the analysis of haplotype frequencies. Geographical subdivision was tested using a contingency χ^2 test of independence (Roff & Bentzen 1989). Wright's fixation index of population subdivision, F_{ST}, was estimated by an analysis of variance of haplotypic frequencies among areas using the AMOVA program of Excoffier. Sequential Bonferroni corrections (Rice 1989) were carried out when multiple comparisons were performed to determine whether individual pair-wise tests were significant on a table-wide basis. We used a modification of Wright's indirect method of estimating gene flow among populations from the level of population subdivision, $Nm = \frac{1}{2}(1/F_{ST}-1)$, where Nm is the effective number of females that migrate between populations per generation (Takahata & Palumbi 1985).

Table 2 Polymorphic sites within mtDNA control region sequences for each haplotype. Light strand reported 5′ to 3′. Position number 16 corresponds to position 15903 of the fin whale *Balaenoptera physalus* mtDNA (Árnason *et al*. 1991)

| Haplotype | | | | | | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 4 |
| | 1 | 1 | 2 | 8 | 9 | 3 | 5 | 9 | 9 | 1 | 3 | 6 | 7 | 8 | 8 | 2 | 9 | 9 | 1 |
	6	9	6	3	7	0	3	2	3	5	3	8	5	1	2	6	0	2	0
1	G	C	A	T	C	C	A	A	T	C	C	A	A	C	C	T	T	T	G
2	.	.	.	.	.	T	.	G	.	.	.	.	G	.	.	.	.	.	.
3	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
4	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.
5	.	.	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.
6	.	.	.	.	.	T	.	G	.	.	.	.	.	.	.	.	C	.	.
7	.	.	.	.	.	T	.	G	.	.	.	.	.	.	T	.	.	.	.
8	.	.	.	.	.	T	.	G	.	.	.	G	.	.	.	.	.	.	.
9	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.
10	.	T	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.
11	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	C	.
12	.	.	.	.	.	T	G	.	.	.	.	.	.	.	T	.	.	.	.
13	.	.	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	.
14	.	.	.	.	.	T	.	.	C	.	.	.	.	.	T	.	.	.	.
15	.	T	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
16	A	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.
17	.	.	.	.	.	T	.	.	.	T	.	.	.	.	T	.	C	.	.
18	.	.	.	.	.	T	.	.	C	.	.	.	T	T	.	.	.	.	.
19	.	.	C	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.
20	.	.	.	C	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.
21	.	.	.	.	.	T	.	.	.	T	.	.	.	.	T	.	.	.	.
22	.	T	.	.	.	T	.	.	C	.	.	.	.	.	T	.	.	.	.
23	.	T	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.
24	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.
25	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.
26	.	.	.	.	.	T	.	G	.	.	.	.	.	.	.	.	C	.	.
27	.	.	.	.	T	T	.	.	.	.	.	.	.	.	T	.	.	.	.
28	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	A
29	.	T	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.

Haplotype	Cook Inlet *n* = 37	Bristol Bay *n* = 24	Norton Sound *n* = 66	Eastern Chukchi Sea *n* = 103	Eastern Beaufort Sea *n* = 94
1	25	–	8	12	–
2	4	–	–	49	10
3	1	–	–	8	–
4	5	–	–	1	–
5	2	22	46	5	9
6	–	–	4	–	–
7	–	–	1	–	–
8	–	–	7	–	–
9	–	–	–	6	49
10	–	–	–	–	1
11	–	–	–	–	1
12	–	–	–	–	1
13	–	–	–	–	4
14	–	–	–	1	9
15	–	–	–	7	–
16	–	–	–	10	–
17	–	–	–	1	–
18	–	–	–	–	1
19	–	–	–	–	1
20	–	–	–	–	1
21	–	–	–	–	2
22	–	–	–	–	3
23	–	–	–	–	1
24	–	–	–	1	–
25	–	–	–	–	1
26	–	1	–	–	–
27	–	–	–	1	–
28	–	1	–	–	–
29	–	–	–	1	–

Table 3 Frequencies of the 29 mtDNA haplotypes in the 5 major beluga whale summering groups

Results

Geographical structure of mtDNA variation

Sequence analysis of 410 base pairs of the 5′ end of the mtDNA control region revealed 19 variable sites defining 29 unique haplotypes among 324 individual whales from five summering grounds (Cook Inlet, Bristol Bay, Norton Sound, eastern Chukchi Sea and eastern Beaufort Sea) and one migration route (animals collected off Point Hope during the northward migration in the spring) (Table 2). Each sampling area was characterized by a few common haplotypes and a greater number of rarer haplotypes (Table 3). Total haplotype diversity was estimated to be 0.844. This is high compared with values for individual locations which range from 0.163 in Bristol Bay to 0.736 in the Chukchi Sea suggesting significant differences in haplotypic composition among areas (Table 1). Overall nucleotide diversity was 0.0051 (0.51%) and varied from 0.0015 in Bristol Bay to 0.0057 in the Chukchi Sea (Table 1). Tajima's test for neutrality provided no evidence of selection acting on this part of the mtDNA control region ($D = 1.83$, $P > 0.05$).

We were unable to reconstruct a comprehensive phylogeny of the 29 haplotypes with maximum parsimony methods due to homoplasy at a number of sites and a limited number of phylogenetically informative sites ($n = 9$). But haplotype relationships could be inferred from a minimum spanning network. The overall frequency of and relationships among the 29 unique sequences are represented on the network in Fig. 2. The main features of this network are a number of star-like phylogenies with several rarer haplotypes linked to a phyletically, more abundant central haplotype. We chose optimal trees from this network using information on haplotype frequency and geographical distribution. For example, in one tree we chose to link haplotype 7 to the more abundant haplotype 5 instead of haplotype 9. Likewise, we linked haplotype 25 to haplotype 5 instead of haplotype 1 (Fig. 2). Haplotype diversity among the five putative stocks is presented in Fig. 3 as a series of one representative tree of a class of most probable trees where highlighted circles represent the presence of a given haplotype in a particular area. Haplotype 5 was the most commonly recorded haplotype and was present in
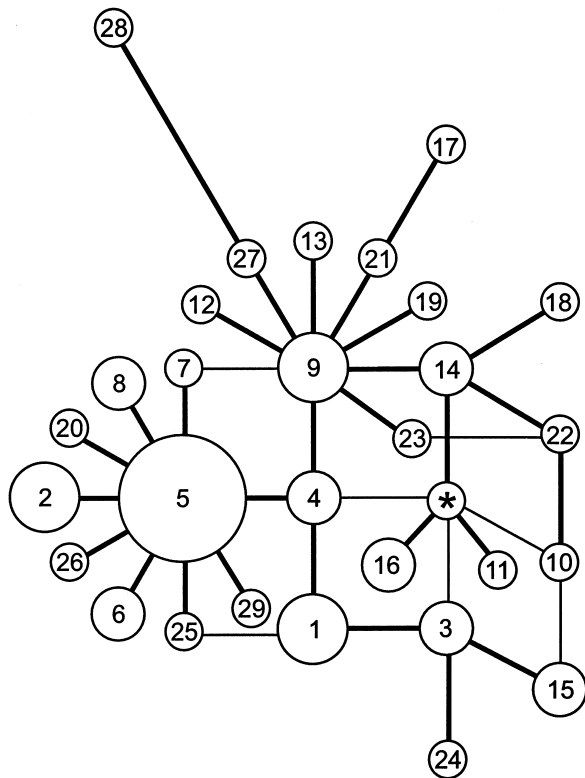
Fig. 2 Minimum spanning network of the 29 unique mtDNA haplotypes. The reconstructed consensus haplotype is denoted by an asterisk. Each link represents a unique mutational event, except in the case of the link between haplotypes 27 and 28 which represents two mutations. Haplotype sizes reflect their abundance in the total sample. Haplotypes are numbered as referred to in the text. A single most parsimonious tree is indicated by the bold lines

all five areas. A number of less abundant haplotypes (e.g. 1, 2, 4 and 9) were found in two or three subpopulations while the majority were rare and typically encountered in only one subpopulation. The haplotypes from particular areas tend to occupy specific portions of the optimal tree. The haplotype distribution shifts from the lower left part of the tree in Cook Inlet to the upper right part of the tree in the eastern Beaufort Sea. This representation of haplotype frequency and interrelationships by area suggests that geographical differentiation is a combination of evolutionary radiation and frequency changes due to drift and gene flow.

The AMOVA revealed a high degree of genetic subdivision among summering locations. Overall, more than 31% of the molecular variance was due to variance among the major summering groups and the migrating whales off Point Hope ($\Phi_{ST} = 0.319$, $P < 0.001$). With one exception pairwise comparisons showed that all five summering concentrations were genetically differentiated from each other at a high level of statistical significance (in all cases $P \ll 0.01$; Table 4). We were unable to discriminate between Bristol Bay and neighbouring Norton Sound ($\Phi_{ST} = 0.019$, $P = 0.127$). Whales caught in the spring off Point Hope were indistinguishable from whales that summer in the eastern Beaufort Sea. In this case however, the former sample was significantly differentiated from the geographically closer summer concentrations in the eastern Chukchi Sea and Norton Sound.

The χ^2 test of haplotype frequencies also revealed that, overall, beluga whales summering in Alaska and northwest Canada are geographically subdivided ($\chi^2 = 641.5$,
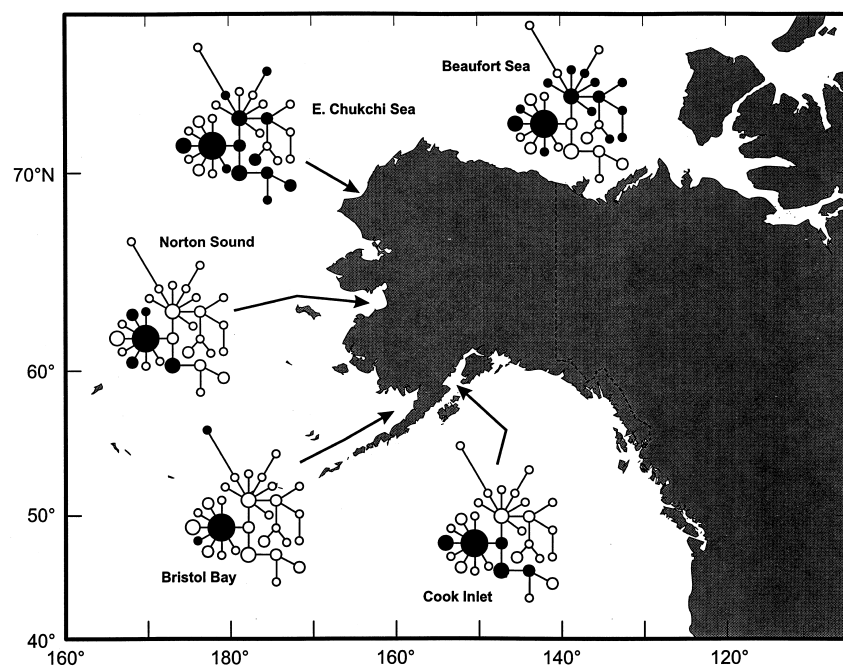
Fig. 3 Haplotype diversity among the five recognized stocks of beluga whales in Alaska and north-west Canada represented on an optimum minimum spanning tree. Solid circles indicate the set of haplotypes in the tree that were found in each stock. Haplotype size reflects overall frequency, not frequency within each area

P ≪ 0.01). All five summering concentrations are significantly differentiated from each other, including Norton Sound vs. Bristol Bay. As with the distance-based statistics (AMOVA), however, we were not able to differentiate among Point Hope and the eastern Beaufort Sea ($\chi^2 = 17.65$, $P = 0.21$). To investigate further whether the discrepancies among the frequency-based (χ^2) and distance-based statistics in our findings on the relationship between Norton Sound and Bristol Bay were due to the type of data used or to some underlying property of the test itself, we analysed the molecular data by the ANOVA format used in AMOVA, this time excluding information on genetic distance among haplotypes. This assumes that all haplotypes are genetically equidistant and the Φ-statistics now become the standard *F*-statistics of Wright (Excoffier *et al*. 1992). As with the other nondistance methods all five summering concentrations were significantly subdivided (overall $F_{ST} = 0.325$, $P < 0.001$), including Bristol Bay from Norton Sound while Point Hope was, as before, indistinguishable from the eastern Beaufort Sea sample (Table 4).

Thus, the molecular genetic analysis agrees very well with our knowledge of movement and seasonal distribution patterns that animals which migrate past Point Hope in spring are part of the same stock that occur in the eastern Beaufort Sea in summer (Fig. 1). Therefore, samples from both locations will be combined in future analyses of the eastern Beaufort Sea stock. A re-analysis of population subdivision following the combination of these two samples did not appreciably alter our findings other than slightly increasing the estimate of overall subdivision ($\Phi_{ST} = 0.329$, $P < 0.001$; $F_{ST} = 0.335$, $P < 0.001$).

Patterns of dispersal and gene flow

Dispersal among summering concentrations, as calculated from the various indices of genetic subdivision, averages one female per generation, indicating that gene flow, although limited, is probably strong enough to prevent fixation of alternative alleles due to random genetic drift (Slatkin & Barton 1989). Estimates of *Nm* for pair-wise comparisons ranged from 0.32 females per generation between Cook Inlet and Bristol Bay to 6.94 between Norton Sound and Bristol Bay (Table 5). Caution is needed however, when estimating gene flow in this manner as patterns of genetic differentiation may be influenced by historical demographic factors as well as by gene flow and drift (see discussion). Furthermore, nonequilibrium between migration and drift may lead to erroneous estimation of gene flow.

The principal limitation in using mtDNA data in studying dispersal patterns is perceived to be its maternal mode of inheritance, i.e. no inferences can be made about male dispersal. We however, disagree for the following reason. Because only those dispersers still living are available for sampling, only recent dispersal events by males can reduce the degree of mtDNA differentiation between perhaps two adjacent populations. In contrast, dispersing females that are living and their mtDNA clonal offspring are available for sampling. Obviously, over time, female dispersal should have a far greater effect on homogenizing mtDNA variation between a population pair than does male dispersal. If mtDNA results indicate that between-population differentiation is greater among females than males, it is conservative to infer that the relative between-population dispersal rate of males is greater than that of females.

		I	II	III	IV	V	VI	VII
Φ_{ST}								
I	Cook Inlet	–	0.00001	0.00001	0.00001	0.00001	*0.00001*	*0.00001*
II	Bristol Bay	0.577	–	0.1269	0.00001	0.00001	*0.00001*	*0.00001*
III	North Sound	0.508	0.019	–	0.00001	0.00001	*0.00001*	*0.00001*
IV	Eastern Chukchi Sea	0.200	0.203	0.210	–	0.00001	*0.00001*	*0.00001*
V	Eastern Beaufort Sea†	0.433	0.444	0.458	0.278	–		
	VI Point Hope	*0.404*	*0.440*	*0.459*	*0.201*		*–*	*0.6823*
	VII Mackenzie–Amundsen	*0.467*	*0.482*	*0.494*	*0.291*		*–0.0107*	*–*
F_{ST}								
I	Cook Inlet	–	0.00001	0.00001	0.00001	0.00001	*0.00001*	*0.00001*
II	Bristol Bay	0.612	–	0.0400	0.00001	0.00001	*0.00001*	*0.00001*
III	Norton Sound	0.425	0.067	–	0.00001	0.00001	*0.00001*	*0.00001*
IV	Eastern Chukchi Sea	0.253	0.441	0.345	–	0.00001	*0.00001*	*0.00001*
V	Eastern Beaufort Sea†	0.361	0.442	0.353	0.212	–		
	VI Point Hope	*0.358*	*0.521*	*0.386*	*0.167*		*–*	*0.6753*
	VII Mackenzie–Amundsen	*0.375*	*0.454*	*0.358*	*0.226*		*–0.0079*	*–*

Table 4 Φ_{ST} and F_{ST} estimates (below diagonal) and significance (*P*) values from 1000 permutations (above diagonal) among the five major summering areas and the spring migration route

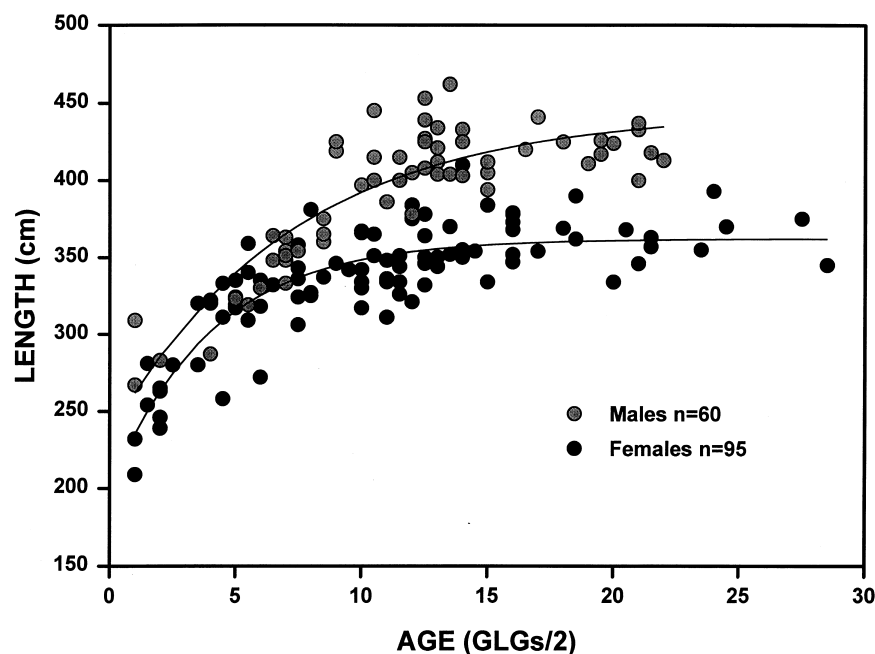†Eastern Beaufort Sea is Point Hope and Mackenzie-Amundsen combined.

Fig. 4 Age-length relationships of 155 beluga whales sampled in north-west Alaska 1988–93. Growth curves were fitted with a Gompertz growth model

For the three northernmost subpopulations, Norton Sound, eastern Chukchi Sea, and eastern Beaufort Sea, we were able to stratify our sample by sex and age. Ages of animals for which only body length data were available were estimated from a standard growth curve (Fig. 4). To reduce the possibility of mother–offspring duplication of genetic data, only adult animals (i.e. females > 4 years, males > 7 years; Burns & Seaman 1986) were used. With those data, females exhibited somewhat greater differentiation among Norton Sound and neighbouring Chukchi Sea subpopulations than did males (Φ_{ST} females = 0.234 vs. Φ_{ST} males = 0.200), and also among Chukchi Sea and adjacent Beaufort Sea (Φ_{ST} females = 0.300 vs. Φ_{ST} males = 0.259), suggesting possible biases in dispersal toward males. When the adult samples from these two locations were further divided into young adult (females > 4 and < 12 years; males > 7 and < 12 years) and older adult (females and males ≥ 12 years) age classes, we found that the older adult cohorts were substantially less subdivided among locations than the younger adult cohorts, particularly for males (Table 6). These analyses suggest that dispersal among the summering concentrations, although limited, is carried out by adult animals, predominantly by adult males.

Discussion

Origins of populations

The phylogeography of mtDNA variants provides important insights into the evolutionary history of contemporary beluga populations. Ultimately, the choice of a single optimal tree or class of trees requires more quantitative analysis. Much of the mtDNA network, however, is resolved and topological differences among MSTs mainly involve only rare haplotypes located at the branch tips

Table 5 Nm values from Takahata and Paulmbi's (1985) G_{ST} estimator modified from Wright's F_{ST}. The eastern Beaufort Sea consists of the Point Hope and Mackenzie-Amundsen samples combined

		I	II	III	IV	V
I	Cook Inlet	–				
II	Bristol Bay	0.317	–			
III	Norton Sound	0.675	6.940	–		
IV	Eastern Chukchi Sea	1.474	0.634	0.950	–	
V	Eastern Beaufort Sea	0.884	0.631	0.916	1.857	–

Table 6 The degree of mtDNA differentiation, Φ_{ST}, between the Eastern Chukchi Sea and Eastern Beaufort Sea beluga whale subpopulations for young and older adult cohorts of males and female. The P-values are from 1000 random permutations of the data matrix

Pairwise comparisons	Φ_{ST}	P
Young females	0.33	0.00001
Old females	0.25	0.00001
Young males	0.33	0.00001
Old males	0.17	0.005

(Fig. 2). The pattern of common, widespread haplotypes connecting to several other population-specific haplotypes may reflect a rapid radiation of a species from an ancestral population into several discrete populations (Lavery *et al.* 1996). This pattern, coupled with the low level of sequence divergence observed in beluga whales compared with other cetacean species (e.g. Baker *et al.* 1993; Dizon *et al.* 1994; Rosel *et al.* 1994), is consistent with a relatively recent expansion of beluga into new habitats following the retreat of the Pleistocene ice sheets. Analyses based on coalescent theory make it possible to identify ancestral haplotypes in intraspecific phylogenies (Crandall & Templeton 1993). Theoretical and empirical studies have shown that older alleles tend to have higher frequencies, occur in the interior of phylogenies, and possess greater numbers of mutational connections (Donnelly & Tavaré 1986; Crandall & Templeton 1993). Furthermore, in subdivided populations ancestral haplotypes are predicted to have the widest distribution among subpopulations (Takahata 1988). The high frequency, widespread distribution, and central position of haplotype 5 in the beluga phylogeny (Figs 2 and 3) suggests that this haplotype was present in the ancestral population and was subsequently distributed among all colonizing summer concentrations once the ice retreated. Since then, population-specific haplotypes have evolved, or through limited gene flow and drift become restricted to individual populations (e.g. haplotype 26 in Bristol Bay, haplotypes 6, 7 and 8 in Norton Sound, haplotype 29 in the eastern Chukchi Sea, and haplotypes 20 and 25 in the Beaufort Sea). Similarly, the relatively high frequency and widespread distribution of haplotype 2 suggest that this haplotype is an interior rather than a terminal haplotype as indicated in the current phylogeny (Fig. 3). Recently we have analysed a number of beluga whales from the Okhotsk Sea in Russia. Some possess unique haplotypes that connect to haplotype 2, while others possess the universal haplotype 5 (G. M. O'Corry-Crowe, unpublished data).

Consideration of the glacial history of the North American continent in conjunction with the current distribution of mtDNA lineages may help determine the origin of present day summer concentrations. Two other studies have examined mtDNA polymorphism in beluga whales in North America. Concentrating on Canadian and Greenland populations, these investigations found that haplotypes were clustered into two distinct lineages, one occurring primarily in animals from eastern Hudson Bay and the Gulf of St Lawrence, and the other predominating in whales from northern Alaska, east to Greenland, and south along the west coast of Hudson Bay (Brennin 1992; Brown 1996). This phylogeographical pattern has also been found in arctic charr *Salvelinus alpinus*, and is consistent with the hypothesis of colonization of Nearctic waters following deglaciation from two glacial refugia in the

Atlantic and Pacific oceans, respectively (Brennin 1992; Brown 1996; Wilson *et al.* 1996). All haplotypes recorded in the present study are part of the latter assemblage suggesting a western centre of origin for Alaskan and northwest Canadian beluga populations.

The chronology of postglacial events (ice shelf retreat, isostatic rebound, and sea level change) was probably highly influential in determining the routes of dispersal from glacial refugia. As the last great ice age came to an end, the Bering land bridge opened along a narrow channel some 11 000 years BP (before present) in what is now the deepest part of the Bering and Chukchi Seas (Denton & Hughes 1981; Elias *et al.* 1996). Beluga probably colonized the ice-free waters of the Beaufort Sea and Amundsen Gulf through this channel. As climate continued to warm, sea level rose and coastlines retreated allowing beluga to colonize Alaska's west coast. This scenario of a somewhat independent origin of the Beaufort Sea population relative to the other Alaskan populations is supported by the star-like phylogeny centered on haplotype 9 (Figs 2 and 3), which may represent a radiation that occurred primarily within the Beaufort Sea. By 9000 years BP, a continuous waterway existed across the Canadian Arctic Archipelago (Denton & Hughes 1981; Dyke *et al.* 1996) enabling beluga to expand their range further eastwards. Subsequent cooling, in concert perhaps with changes in oceanographic circulation, closed this channel. Throughout the remainder of the Holocene, beluga whales along with other Arctic marine fauna, including the bowhead whale *Balaena mysticetus*, may have witnessed other range expansions and contractions in response to climatic changes (Dyke *et al.* 1996). Nevertheless, throughout the postglacial period, winter ice maxima most likely necessitated the seasonal retreat by belugas to lower latitudes such that today in the Beaufort Sea and over much of the species range beluga undertake seasonal movements between long-established summering areas at high latitudes and wintering grounds at the edge of the pack ice.

Geographical subdivision

Analysis of variation within the mitochondrial genome revealed substantial levels of genetic subdivision among summer concentrations of beluga whales in the western Nearctic. The average level of genetic differentiation among the five geographical subpopulations ($\Phi_{ST} = 0.33$) (i) is similar to that found among seasonal concentrations of narwhal *Monodon monoceros* in the north-west Atlantic Ocean (H_{ST} ranged from 0.24 to 0.73; Palsbøll *et al.* 1997); (ii) is greater than that found among geographically distinct subpopulations of harbour porpoise *Phocoena phocoena* in the north-east Pacific Ocean ($\Phi_{ST} = 0.18$; Rosel 1992 and additional unpublished data from our laboratory), and (iii) is more than twice that observed between

populations of short-beaked common dolphins *Delphinus delphis* in the Black Sea and eastern tropical Pacific Ocean ($\Phi_{ST} = 0.15$; Rosel 1992) and among populations of striped dolphins *Stenella coeruleoalba* from the Atlantic and Pacific Oceans ($\Phi_{ST} = 0.14$; Archer 1996).

Cook Inlet is the most genetically distinct of all geographical subpopulations with respect to mtDNA, an indication perhaps that the Alaska peninsula may indeed be an effective barrier to genetic exchange or that drift in this small population offsets any homogenizing effects of gene flow. Although no obvious geographical barriers exist between the remaining summer concentrations, all were found to be genetically differentiated from each other. The limited mtDNA subdivision detected among Norton Sound and Bristol Bay is probably more a consequence of demographic history than contemporary gene flow. Each possess unique haplotypes and the high frequency of haplotype 5 in both areas may represent close historical ties between the two (Table 3). One, for example, may have been founded from the other. The relatively small number of samples from Bristol Bay also severely limits the power of our analysis to detect differentiation (Peterman 1990; Dizon *et al.* 1995). The discrepancy between the frequency-based and distance-based analyses regarding the relationship between Norton Sound and Bristol Bay, however, raises an important question over the choice of the correct statistic in the analysis of geographical subdivision (see also Hudson *et al.* 1992). In situations where there is little or no phylogeographical structure and each haplotype does not differ by much from all other haplotypes, as in the Norton Sound–Bristol Bay comparison, average interhaplotypic differences within populations may not differ significantly from average differences between populations, even if there are significant differences in haplotypic frequency between populations. Under these circumstances the choice of a distance-based analysis may act to conceal rather than resolve population structure.

Although the failure to discriminate between the Point Hope and eastern Beaufort Sea samples does not, in itself, prove that they are both from the same population (Dizon *et al.* 1995), the molecular genetic analysis supports our limited knowledge of the movement and seasonal distribution patterns of animals which migrate past Point Hope in spring and that these are part of the same stock that occur along the eastern Beaufort Sea coastline in summer (Fraker 1980; Moore *et al.* 1993).

Maternally directed philopatry

In light of the limited significance of geographical barriers and the long-distance dispersal capabilities of beluga whales, the mtDNA population structure indicates strong philopatry to discrete summering areas. Such site fidelity is particularly impressive considering the possibility that some subpopulations may overwinter in a common area. Maternally directed philopatry to seasonal habitats, as opposed to physical barriers to movement, has been identified as a major influence on population genetic structure in a number of other highly migratory species, including marine turtles (Meylan *et al.* 1990; Bowen *et al.* 1992), shore birds (Wenink *et al.* 1993) and great whales (Baker *et al.* 1994; Palsbøll *et al.* 1995; Larsen *et al.* 1996). There are probably selective advantages in returning to a familiar habitat with predictable food resources, climatic, and oceanographic conditions. The prolonged period of association between mother and offspring in many cetacean species may aid in the establishment of homing behaviour to seasonal habitats and facilitate in the development of matrilineal population structure. In humpback whales, the cultural transmission of migratory destinations is thought to occur when calves complete a round-trip migration between feeding grounds and natal wintering grounds before separating from their mothers (Martin *et al.* 1984; Baker *et al.* 1994). Significantly perhaps, beluga whale calves may complete two, possibly three, migratory circuits between summering grounds and wintering areas in association with their mothers, and at least one maternal sibling (Smith *et al.* 1994).

Management implications

Geographical subdivision at the mtDNA locus, however, does not necessarily mean that geographical populations are genetically isolated because male-biased dispersal and gene flow may limit or eliminate population structure at nuclear loci (Karl *et al.* 1992; Palumbi & Baker 1994; Avise 1995). An understanding of the male contribution to genetic exchange awaits completion of analyses of microsatellite polymorphisms. Nevertheless, from an ecological perspective, mtDNA can provide important information on how to define biologically meaningful management units. Because recruitment is dependent on female reproductive success, limited female dispersal results in demographically independent populations, irrespective of whether there is extensive male dispersal or gene flow (Moritz 1994; Avise 1995). One prediction of this matrilineal population structure is that colonization of depleted populations will be slow due to limited recruitment from outside females. This has been recorded in green turtles, *Chelonia mydas* (see Bowen *et al.* 1992) and is consistent with the failure of some beluga summer concentrations to recover following near extirpation more than a century ago (Reeves & Mitchell 1987, 1989).

Despite the considerable level of geographical subdivision, the lack of strong phylogeographical structure in the mtDNA variation suggests a close evolutionary

relationship between the discrete geographical subpopulations (Avise *et al.* 1984; Neigel & Avise 1986). Thus, our findings suggest that the five recognized summer concentrations of beluga whale in Alaska and north-west Canada are demographically, if not evolutionarily, distinct subpopulations and should be treated as separate management stocks for monitoring population trends and designing management policies that maintain population and species viability.

Patterns of dispersal and gene flow

The method used to estimate average levels of gene flow (*Nm*) among subdivided populations was initially developed for an island model (Wright 1951) but has been found to be consistent over a wide range of assumptions about population structure, selection, and drift (Slatkin & Barton 1989). All the models tested, however, assume equal population size among subpopulations and nonoverlapping generations, conditions that rarely occur in the wild. Nevertheless, Takahata & Palumbi's (1985) modified *Nm* gives us an idea of the order of magnitude of female dispersal required to maintain the degree of subdivision observed.

Most values of *Nm* were less than 1.0, suggesting that female dispersal among beluga whale populations was low. Despite its uniparental mode of inheritance, we show it may be possible to determine relative rates of male and female dispersal using mtDNA data. Even when sex differences exist in life-history parameters (e.g. birth sex-ratio and survival), as long as these differences are similar for residents and immigrants, only gender-biased dispersal can result in differences in the ratio of immigrant vs. resident haplotypes for one sex compared with that for the other. However, despite exploring various resampling approaches we have yet to find a statistical procedure that adequately tests for significant differences in Φ_{ST}s among male vs. among female cohorts. Because there is much overlap in haplotype composition among neighbouring subpopulations, many dispersal events go undetected by the genetic differentiation (Φ_{ST}) approach. Considering this method's limited statistical power in these situations, only considerable differences in dispersal rates between the sexes would be expected to result in detectable differences in levels of mtDNA differentiation (Φ_{ST}) of one sex compared with that of the other. Because differences in Φ_{ST}s are apparent in spite of the anticipated low statistical power, we thus propose that dispersal among summer concentrations of beluga, although limited, was predominantly by adult males, and the lower differentiation recorded among older compared with younger adults may mean that older age cohorts disperse more or that the probability of dispersing increases with age. Hopefully, in the future, this can be formally tested.

The timing of dispersal is unknown. Our samples were collected 2–6 months after the peak of the breeding season, indicating that dispersal lasts at least through post breeding. This picture of probable male-biased dispersal in beluga whales contrasts with that for long-finned pilot whales *Globicephala melas* (Amos *et al.* 1993), and resident forms of killer whale *Orcinus orca* (Bigg *et al.* 1990), where both males and females are considered to remain within their natal pods for their entire lives. This asymmetry may be associated with the type of mating system. In species where females benefit from being philopatric and remaining near close kin, males increase their access to mating opportunities and avoid maladaptive levels of inbreeding by dispersing (Greenwood 1980; Shields 1987). Greenwood proposed that such male-biased dispersal was characteristic of a mate-defence mating system in contrast to a resource-defence mating system where males set up and defend territories around important resources to attract mates. These hypotheses, however, were developed from a review of terrestrial species. The three-dimensional nature of the marine environment, and unpredictable distribution of many of its resources provides little or no possibility of defending resources (but see below) and offers unique challenges to mate defence (Clapham 1996), particularly if mating occurs during migration (Clutton-Brock 1989).

Differences among the sexes in other traits, such as body size and the level of parental investment may also be strongly associated with the type of mating system. Although sexual size dimorphism in mammalian species may evolve in a number of ways (Ralls 1976, 1977), the larger size of adult male beluga whales compared with females (Sergeant & Brodie 1969; Burns & Seaman 1986; Doidge 1990; Fig. 4) may be a consequence of a polygynous mating system where body size is a more critical determinant of breeding success in males (Clutton-Brock 1989, 1994). Similarly, the segregation of adult male beluga whales from adult females, subadults, and juveniles following breeding (Kleinenberg *et al.* 1964; Ognetev 1981; Caron & Smith 1990; Smith *et al.* 1994) suggests an absence of male parental care in this species, a characteristic that may be related to more intense competition in males for access to mates (Ralls 1977; Clutton-Brock 1989, 1994).

Many aspects of beluga mating systems have yet to be elucidated. For example, the potential for sperm competition and defence of predictable resources outside the mating period (e.g. molting sites), and the importance of female choice have yet to be assessed. Nevertheless, the difficulties associated with defending a mate in the marine environment, especially during migration, along with the sex differences in body size, the level of parental investment, and possibly dispersal observed in the beluga whale are consistent with some form of polygamous mating system where males compete directly for access to mates.

Acknowledgements

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