POPULATION GENETICS OF HUDSON BAY BELUGA WHALES

POPULATION GENETICS OF HUDSON BAY BELUGA WHALES (*Delphinapterus leucas*): AN ANALYSIS OF POPULATION STRUCTURE AND GENE FLOW USING MITOCHONDRIAL DNA SEQUENCES AND MULTILOCUS DNA FINGERPRINTING

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ABSTRACT

Beluga whales in Canadian waters are subdivided into at least six genetically distinct stocks maintained by geographic separation and philopatry to estuaries in summer. Belugas in eastern and western Hudson Bay have previously been shown to be compose genetically distinct populations using mitochondrial restriction analysis. It is not known whether these stocks are further subdivided on the basis of specific estuarine use. Mitochondrial DNA control region sequences were used to investigate variation among belugas sampled at several sites along eastern Hudson Bay, Hudson Strait and Ungava Bay. 320 bp were sequenced, including the highly variable 5' region of control region, in 126 belugas. 17 variable sites and 17 haplotypes, which clustered into 2 related groups, were detected among the whales sequenced. Haplotypes of group A were found mostly in eastern Hudson Bay sites, while B group haplotypes were predominant in northern populations. Significant differences in frequencies of haplotype groups were found between eastern Hudson Bay and Southern Hudson Strait/Ungava Bay populations, indicating they are genetically distinct populations. Haplotype distribution patterns also suggested possible differences between belugas using different estuaries along eastern Hudson Bay. The presence of both groups in each population indicated some exchange of individuals between populations, and/or between eastern and western Hudson Bay.

Multilocus DNA fingerprinting was used to investigate the extent of gene flow between eastern and western Hudson Bay belugas via interbreeding on common wintering grounds in Hudson Strait. Belugas from St. Lawrence estuary and the Mackenzie Delta were also analyzed to measure their genetic relatedness to Hudson Bay whales as well as for purposes of comparison to earlier fingerprinting analyses. While results supported lower genetic diversity within the St. Lawrence population, the range of bandsharing within and between populations was otherwise low (0.09 - 0.17 for Jeffreys 33.15 and 0.12 - 0.22 for Jeffreys 33.6). Mantel tests showed differences among St. Lawrence, Hudson Bay, and Mackenzie Delta populations, but not within Hudson Bay. The conflicting nature of the data did not allow conclusions regarding gene flow. Therefore, DNA fingerprinting was not considered to have provided sufficient resolution in addressing this issue.

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GENERAL INTRODUCTION

A central requirement in the conservation and management of cetacean species is the ability to define stocks or management units (MU's; Moritz 1994). Stocks can be described as populations which are geographically or behaviourally independent so as to be genetically distinct (Moritz 1994; Lande 1991). Genetic analysis can be extremely useful in determining the identity of stocks to be managed, as it may detect differences between populations not apparent from studies of morphology, distribution, or behaviour.

Mitochondrial DNA is well suited for this purpose due to its rapid mutation rate (Brown *et al.* 1979; Wilson *et al.* 1985) and haploid, maternal inheritance which results in an increased sensitivity to genetic drift (Birky *et al.* 1989). Identification of stocks is best accomplished by detecting significant divergence in allele frequencies, regardless of phylogeny of alleles, because allele frequencies will respond to population isolation more rapidly than phylogeographic patterns (Niegel and Avise 1986; Lande 1991). Variable alleles can remain in a population for periods of time long enough for significant divergence to occur (Gyllenstein and Erlich 1989; Wayne *et al.* 1990). Cetaceans are highly mobile animals, but at the same time exhibit varying levels of site fidelity and population subdivision. Thus, among cetacean species there are broadly varying patterns of diversity. Mitochondrial DNA has been used in many studies to identify and evaluate these

patterns.

As is the case for many terrestrial mammals, population structure for some cetaceans is defined by physical barriers, usually in the form of large land masses such as continents. High mtDNA divergence is often found between populations in different oceans (e.g. bottlenose dolphins, Dowling and Brown 1993; humpback whales, Baker *et al.* 1993; killer whales and minke whales, Hoelzel *et al.* 1991; Hoelzel and Dover 1991b).

Genetic differences between populations can also occur in the absence of obvious barriers. Differential habitat use by different pods of whales or different morphometric forms can effectively isolate them to the extent that divergence between them can be as great as that between allopatric populations (Wada and Numachi 1991; Hoelzel and Dover 1991a; Rosel 1994). Alternatively, divergence between sympatric populations can occur through social cohesion of matrifocal pods despite considerable intergroup movement of males (e.g. in pilot whales, Amos *et al.* 1991, 1993; bottlenose dolphins, Duffield and Wells 1991).

Populations of cetaceans can remain genetically divergent even in the face of considerable migration, due to the high degree of philopatry to nursery and feeding areas. Philopatry exhibited by females and passed on to offspring can result in maintenance of genetic differences between populations as well as further divergence due to drift. This has been observed in species such as right whales and humpbacks, which migrate over large distances but also show a considerable amount of site fidelity, and therefore show low mitochondrial gene flow among populations (Baker *et al.* 1990, 1993; Schaeff *et al.* 1993).

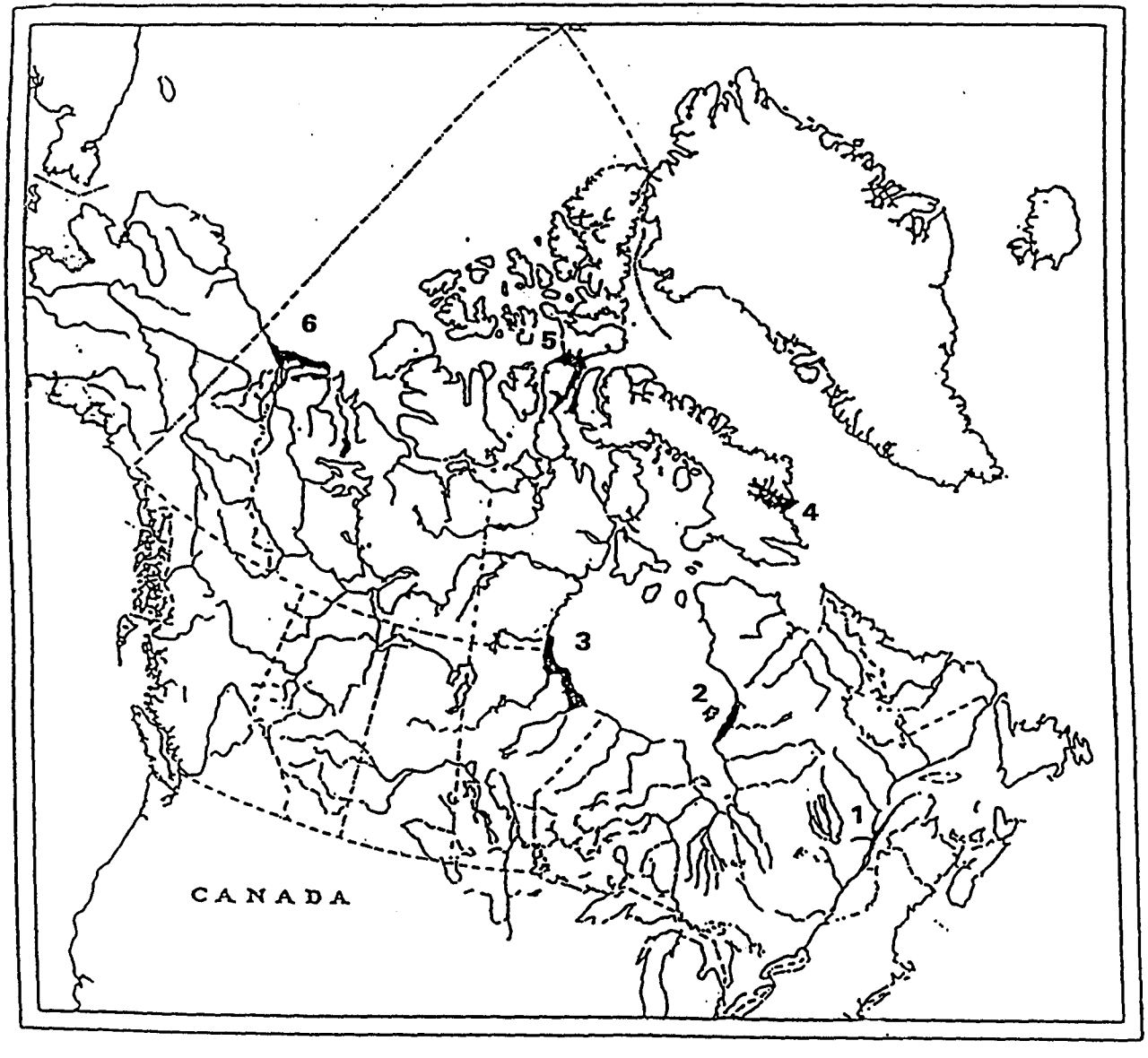
Information on population structuring inferred only from mitochondrial

DNA studies, however, is not enough to make an informed decision about the genetic relatedness of populations. Valuable data on male-mediated gene flow is not available from maternally inherited mtDNA. A genetic definition of a stocks should be based upon information from both mitochondrial and nuclear DNA analyses. Most data from ncDNA markers to date have been used to detect or confirm differences between populations of cetaceans in studies comparing allozyme frequencies (Danielsdottir *et al.* 1991; Wada and Numachi 1991; Wada *et al.* 1991) and minisatellite bandsharing levels (van Piljen *et al.* 1995). However, genetic exchange through males moving between populations or interbreeding may result in ncDNA patterns which may not match mtDNA patterns (Karl and Avise 1992; Karl *et al.* 1992).

Beluga whales inhabiting arctic and subarctic waters are subdivided into six populations based on observations of morphometrics and geographic discontinuity (Table 1): Cumberland Sound/Southeast Baffin, eastern Hudson Bay, western Hudson Bay, high Arctic, Beaufort Sea and the St. Lawrence (Sergeant and Brodie 1975). Several of these populations were drastically reduced in number through commercial harvests (Brodie *et al.* 1981; Mitchell and Reeves 1981; Reeves and Mitchell 1984, 1987a,b). Two of these populations, the St. Lawrence and Southeast Baffin stocks, are listed as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC).

One further population, that in the eastern Hudson Bay, is listed as threatened. Mitochondrial DNA evidence indicates that eastern and western Hudson Bay belugas belong to genetically distinct stocks (Brennin 1992; Brennin *et al.*, in prep.), such divergence likely originating due to separation for about

FIGURE 1. Map of Canada showing distributions of the six recognized stocks of beluga whales in Canadian waters. 1 = St. Lawrence estuary; 2 = eastern Hudson Bay; 3 = western Hudson Bay; 4 = southeast Baffin/Cumberland Sound; 5 = high Arctic; 6 = Mackenzie Delta. (Modified from Pippard 1985).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CANADA

50,000 years during the Wisconsin Ice Age, and maintained by female-directed philopatry to summering estuaries. Belugas in eastern Hudson Bay are still harvested by Inuit during their summer occupation of estuaries. The establishment of quotas in Inuit communities indicates a recognition of the need to manage belugas along the coast of Nunavik (Northern Quebec).

The aim of the first part of this study was to further define the genetic relationships of whales within the eastern Hudson Bay population. Currently it is not known whether each of the beluga populations comprise a single stock, or are further divided into a number of stocks which use one or more estuaries in a given area (Reeves and Mitchell 1987a). To address this question, a highly variable portion of the mitochondrial DNA control region was sequenced in 126 belugas from 12 sampling sites along the Nunavik coast and haplotype frequencies were compared among populations.

The objective of the second part of this thesis was to assess the degree to which gene flow may be occurring between the eastern and western Hudson Bay stocks. These summering stocks are believed to share a common breeding ground during the winter months. The hypothesis that these two stocks comprise a single breeding population was tested using DNA fingerprinting. For this study, Hudson Bay stocks were compared to each other and to the St. Lawrence and Mackenzie Delta populations, two populations most likely to differ based on their nonoverlapping distributions and long period of separation.

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INTRODUCTION

Beluga whales (*Delphinapterus leucas*) inhabit all arctic and subarctic waters, having a circumpolar distribution. Adaptations to arctic habitats include thick skin, increased dive duration, and the loss of a dorsal fin and accompanying development of thick dorsal ridge (Kleinenberg 1964). The seasonal distribution of belugas is influenced primarily by ice cover. Movements are restricted to areas of partial ice cover, as open water is avoided due to killer whale (*Orcinus orca*) predation. Belugas also avoid high to complete ice cover, with its attendant dangers of polar bear predation (Smith 1985; Lowry *et al.* 1987a,b) and ice entrapment (Mitchell and Reeves 1981).

Complete ice cover can therefore act as a physical barrier which can limit the range of the species, or separate populations over geologic time. Belugas on the eastern and western coasts of North America were completely separated for approximately 50,000 years by the Laurentide ice sheet during the Wisconsin ice age (Denton and Hughes 1981). Such isolation allows the genetic composition of populations to diverge as forces of random genetic drift and selection work on them independently. Such differences will remain and increase if contact between populations is not re-established upon removal of the barrier (Slatkin 1989).

In addition to the physical geographic influences on beluga population structure, behavioural factors also act to determine distributions of belugas. An

important feature of beluga life history involves the preferential use of estuaries in summer. Recent work has shown that the warmer waters and abrasive surfaces of estuaries promote the active shedding of old skin and growth of new layers (St. Aubin and Geraci 1988, St. Aubin *et al.* 1990). The use of estuaries by females and their calves probably contributes to mother-calf bonding, as calves are highly dependent on mothers during the lactation period, which lasts 1.4 to 2.7 years (Brodie 1971; Sergeant 1973; Doidge 1990). Over this period, calves have ample opportunity to learn migration routes and other behaviours.

Behavioural observations have shown that belugas will return to an estuary within days or even hours following a disturbance, despite intense boat traffic or hunting pressure (Finley *et al.* 1982; Caron and Smith 1990). Further, this preference persists from season to season, as belugas have been recorded to return to the same estuaries in different years (Caron and Smith 1990). Such site fidelity, or philopatry, to feeding, breeding or nursery areas is widely observed in cetacean populations (e.g. in humpbacks, Clapham and Mayo 1990, Baker *et al.* 1990, 1993; right whales, Schaeff *et al.* 1993).

Strong levels of philopatry can maintain the separation of populations in the absence of physical barriers, by restricting movement of individuals between populations. This appears to be the case with belugas which recolonized the eastern and western shores of Hudson Bay after recession of the ice sheet. Despite 7,000 years without physical barriers to prevent mixing of summering populations, mitochondrial RFLP analysis showed that whales captured in estuaries along eastern Hudson Bay are genetically distinct from those in western Hudson Bay rivers (Brennin 1992; Brennin *et al.*, in prep.).

The eastern Hudson Bay and Ungava Bay populations were severely reduced by commercial harvesting (Reeves and Mitchell 1987a,b). Inuit harvesting continues, and therefore it is important to determine the extent of genetic distinction between these and other populations in order to more accurately define the resource from which harvesting draws.

The use of mtDNA in population genetic studies has become increasingly common over the past two decades. Mitochondrial DNA studies of relatedness of populations, species, and higher taxa have been carried out in a wide variety of mammalian taxa including bears (Cronin *et al.* 1991a; Shields and Kocher 1991; Taberlet and Bouver 1994), primates (Hasegawa *et al.* 1990; Disotell *et al.* 1992; Hoelzer *et al.* 1994), canids (Wayne *et al.* 1990, 1991; Lehman *et al.* 1992; Geffen *et al.* 1992), cervids (Miyamoto *et al.* 1990; Cronin *et al.* 1991b; Cronin 1991, 1992), felids (Menotti-Raymond and O'Brien 1993), and rodents (Plante *et al.* 1989a,b; Thomas *et al.* 1990; Smith and Patton 1993).

Similarly, mtDNA analysis has been used to study many cetacean species. Most of this work has involved the use of restriction fragment length polymorphisms (RFLP's) to investigate stock distinction, migration patterns, introgression, and sympatry (Dizon *et al.* 1991; Hoelzel and Dover 1991a,b; Baker *et al.* 1990,1993; Schaeff *et al.* 1993).

Mitochondrial DNA has many properties which distinguish it from nuclear DNA (ncDNA). It is inherited from the maternal parent only (Dawid and Blacker 1972, Hutchison *et al.* 1974), with low levels of paternal leakage (Gyllenstein *et al.* 1985, but see Hoeh *et al.* 1991). It does not undergo recombination and thus exists as a haploid genome. Secondly, mtDNA has a relatively rapid rate of

sequence evolution, approximately 5-10 times that of single copy nuclear DNA (Brown *et al.* 1979; Cann and Wilson 1983; Wilson *et al.* 1985). The mutation rate of the mtDNA genome of mammals was estimated to be 2%/Myr (Brown *et al.* 1979, 1982), from studies on primates and rodents. Similar estimates have been reached in other studies of other primates (Wilson *et al.* 1985; Cann *et al.* 1987), rodents (Ferris *et al.* 1983), and artiodactyls (Upholt and Dawid 1977). However, this rate may be slower in cetaceans (Hoelzel *et al.* 1991; Baker *et al.* 1993, Martin and Palumbi 1993).

The features described above make mitochondrial DNA useful for assessing genetic diversity and population structure. The maternal mode of inheritance allows patterns of migration and colonization in species with maternally-focused distributions to be traced without complications arising from patterns of dispersal by males. The lack of recombination allows mtDNA genotypes to persist over generations, providing clear definitions of maternal genealogies. Further, because it is inherited uniparentally and therefore haploid, the mtDNA has an effective number of alleles which is one-fourth that of nuclear genes (Birky *et al.* 1989). Therefore, loss of diversity and differentiation of isolated populations due to genetic drift will have a greater impact on mitochondrial than on nuclear markers. Mitochondrial DNA should therefore be a sensitive indicator of female-mediated gene flow and founder effects, showing greater differences than nuclear markers between demes (Birky *et al.* 1989; Takahata and Palumbi 1985), particularly where females are more sedentary than males (e.g. in humpback whales, Baker *et al.* 1993).

Studies of RFLP's yield information at a relatively coarse level of

resolution. Studies involving differentiation for periods over shorter time scales (50,000 years or less) may be beyond the scope of RFLP analyses (Palumbi *et al.* 1991). Restriction analysis is further limited by the need for relatively fresh tissue from which circular mtDNA can be extracted. This is not always possible for studies of free-ranging animals such as cetaceans.

These problems have been overcome through the development of methods of direct sequencing of DNA segments amplified by the polymerase chain reaction (PCR) (Saiki *et al.* 1988). PCR-based analyses can be performed on small quantities of DNA such as those recovered from biopsy sampling of free-ranging cetaceans (Lambertsen *et al.* 1987; Patenaude and White 1995), as well as on highly degraded DNA from beached animals.

Analysis of DNA sequences allows observation of exact base position changes, as well as permitting analysis of more variable portions of the mtDNA genome to be surveyed. It is thus considered to provide higher resolution data than RFLP data. One such region is the control region, which is involved in the regulation of replication of the heavy strand of the mtDNA molecule (Clayton 1982). Analysis of this noncoding region have shown mutation rates 3-5 times higher than the rest of the mtDNA genome (e.g. Aquadro and Greenburg 1983; Vigilant *et al.* 1989). Consequently, sequence analysis of the control region should reduce the time span over which genetic differences can be detected (Vigilant *et al.* 1989). Several studies comparing the two methods found greater levels of diversity uncovered by sequences of the mitochondrial control region (e.g. Wayne *et al.* 1990; Brown *et al.* 1993; Lamb *et al.* 1994). Baker *et al.* (1993) measured 10x greater nucleotide diversity among humpbacks whales using

control region sequences than with RFLP's (Baker *et al.* 1990). Surveys of finer-scale geographic structuring might therefore be better served by comparisons of sequences of highly variable regions of the mtDNA genome.

The study described in this chapter was aimed toward identifying the boundaries of stock identity within the eastern Hudson Bay and Southern Hudson Strait populations using sequence analysis of the mtDNA control region. Analysis of RFLP haplotypes among Hudson Bay belugas (Brennin 1992; Brennin *et al.*, in prep.) found geographic segregation of two major lineages of haplotypes between eastern and western Hudson Bay, as populations in the east were composed mostly of one lineage, while the other lineage was predominant in the west. These data support the presence of two genetically distinct stocks within Hudson Bay. However, it is not presently clear whether beluga summering stocks comprise continuously distributed groups of whales, or whether they are further subdivided into genetically distinct smaller stocks defined by specific estuarine use (Reeves and Mitchell 1987a). Additionally, whales summering in southern Hudson Strait have not been analyzed previously for mtDNA variation. These belugas were sequenced to determine their relationship to eastern Hudson Bay and Ungava Bay animals.

MATERIALS AND METHODS

Sample Collection

Beluga tissue samples were collected over several years from Inuit kills at various locations in eastern Hudson Bay, Hudson Strait, Wakeham Bay, and Ungava Bay with the co-operation of Makivik Corporation and the Department of Fisheries and Oceans (Fig 1.1). Eight samples in eastern Hudson Bay were collected from animals captured during satellite tagging studies. All tissues (skin, heart, muscle, liver and kidney) were shipped frozen or preserved in a NaCl-saturated solution containing 20% DMSO and 0.25 M EDTA (pH 8.0) (Seutin *et al.* 1991).

DNA Extraction and Quantification

Tissue samples (0.50-0.75g) were ground to a fine powder in liquid nitrogen and suspended in 3.5 mL lysis buffer (5 M urea, 0.2 M NaCl, 0.1 M Tris-HCl, pH 8.0, 0.5% *n*-laurylsarcosine, 10 mM EDTA), and incubated for one to three weeks at 37°C. During this time samples were subjected to digestion by Proteinase K (83 units), either twice at 37°C for 12 h, or once at 55°C for 90 min followed by 12 h at 37°C. DNA was extracted twice with phenol and chloroform (70:30) and once with chloroform, and then precipitated by adding sodium acetate to a final concentration of 0.15 M and two volumes of cold 95% ethanol. DNA

pellets were washed with 70% ethanol, air-dried and dissolved in 0.5-1.0 mL TNE_2 (10 mM Tris-HCl, 10 mM $NaCl_2$, 2 mM EDTA, pH 8.0) overnight at 37°C.

Quantity of DNA was determined using TKO 100 DNA Mini-Fluorometer (Hoefner), which assays DNA concentration based on specific binding of Hoechst 33258 fluorescent dye to A-T rich regions of DNA (Cesarone *et al.* 1979). This estimate was then adjusted by running 1 μg of *Eco*RI-digested DNA in agarose gels and comparing to a standard of known concentration. Gels were stained with ethidium bromide and visualized under shortwave UV light.

Gender Determination

Gender determination was performed for those samples not so classified at the time of collection via the PCR-ZFY/ZFX method of Palsbøll *et al.* (1992). Amplification was carried out using primers ZFY1204 (5'-CATTATGTGCTGGTT CTTTTCTG-3') and ZFY0097 (5'-CATCCTTTGACTGTCTATCCTTG-3'). Reactions contained 250 ng DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.01% gelatin, 1 unit of Amplitaq (*Thermus aquaticus*) DNA polymerase (Perkin Elmer), 200μM each dNTP, and 0.5 μM of each primer. Thermocycle profiles consisted of 1 min at 94°C, 1 min at 60°C, and 4 min at 72°C, with a 5 min extension at 72°C in the final cycle to ensure complete amplification of products. Products were digested with *TaqI* restriction enzyme (Bethesda Research Laboratories) run on 2% LMP agarose minigels in TBE buffer (45 mM Tris-borate (pH 8.0), 1 mM EDTA) at constant voltage (75V) for 4 to 5 h. DNA was visualized as described above.

Amplification of mtDNA Control Region

A 1 kb region of the control region was amplified using the universal control region primers L15296 (5'-TCAAAGCTTACACCAGTCTTGTAAACC-3') and H00651 (5'-TAACTGCAGAAGGCTAGGACCAAACCT-3') (Kocher *et al.* 1989). Numbers refer to the 3' base of the oligonucleotide with reference to the human sequence of Anderson *et al.* (1981). Amplification reactions (25 μL) contained 250 ng DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 1 unit of Amplitaq (*Thermus aquaticus*) DNA polymerase (Perkin Elmer), 200μM each dNTP, and 0.5μM each primer. Thermal conditions consisted of 1 min at 94°C, 1 min at 50°C, and 1.5 min at 72°C for 30 cycles, with a 5 minute 72° hold to ensure complete extension of PCR products.

Amplification products were loaded into 1% LMP agarose (Sigma) minigels which were run for 3.5 to 4 h at constant voltage (70V) in TAE (40 mM Tris-acetate, 3 mM sodium acetate, 1 mM EDTA, pH 7.8). Gels were then stained with ethidium bromide and DNA visualized under shortwave UV light. A single band approximately 1 kb in size was excised and placed in a 1.5 mL microfuge tube containing 250-300 μL sterile distilled dH_2O to allow elution of DNA from the gel slice. Ten μL of this solution was used in a second amplification (50 μL) using the L15296 primer and a nested primer designated Phh (5'-CCATCTAGA CATTTTCAGTG-3') (R.W. Lillie, personal communication) under the same conditions as described above. Two reactions were carried out for each initial product. Six to seven μL of the reaction products were run on 1% agarose gels at 100-120V in TBE buffer for 1.5 to 2 h, and viewed as above.

Purification and Sequencing of Control Region PCR Products

Those samples yielding two single-product reamplifications of sufficient quantity were combined and extracted at least once with phenol and chloroform (70:30) and once with chloroform. DNA was precipitated by addition of ammonium acetate to a final concentration of 1.3 M and 1.5 volumes of isopropanol, then storage overnight at -20°C. The DNA precipitate was pelleted out by microfugation at high speed for 30 min, then washed with 70% ethanol, air-dried and dissolved in 15 μL sterile dH_2O. DNA was quantified by the Hoechst 33258 binding assay using the TKO 100 Mini-Fluorometer (Hoefner) and diluted when necessary to give a concentration of 30 ng/μL.

PCR Cycle Sequencing using the ABI 373A Automated Sequencer (Applied Biosystems, Inc.). Thermocycle conditions consisted of 30 sec at 96°C, 15 sec at 50°C, and 4 min at 60°C for 25 cycles and utilized fluorescently labelled terminators. Sequencing of the light strand of the mtDNA control region used a primer designated Bel 5' (5'-ACATTTTACTGTGACTATTG-3') (W.R. Lillie, pers. comm.) which consists of base positions 70 to 92 on the light strand of the control region. A second primer, WWOS (5'-GAGATATGGGCCCGGTGCGAG-3') was designed to sequence the heavy strand beginning from base position 371.

Sequence Analysis

Sequences were aligned and edited using ESEE sequence editor (Cabot and Beckenbach 1989). Editing involved comparisons of sequences generated from each strand along with consultation of the sequence printout. Variable sites were identified and used for the assignment of haplotypes.

Haplotypes were aligned to outgroups *Phocoena phocoena* (Rosel 1992) and *Orcinus orca* (Hoelzel *et al.* 1991) using CLUSTALW (Thompson *et al.* 1994). Phylogenetic analyses of the aligned sequences were performed using the neighbour-joining and maximum-likelihood algorithms in the PHYLIP package (Felsenstein 1993). Statistical significance was tested using the SEQBOOT bootstrap resampling procedure included in the package.

Indices of nucleotide diversity (π) and haplotype diversity (h) were calculated as in Nei (1987, p. 256) and Nei and Tajima (1981), respectively. These indices were calculated for several possibilities of population subdivision.

Population Comparisons

Genetic divergence between populations (d_{XY}) was determined and corrected for intrapopulation diversity (d_A) by the method outlined in Nei (1987).

Measures of population subdivision at the nucleotide level (N_{ST}) were calculated as outlined in Lynch and Crease (1990). This analogue of Nei's (1973) G_{ST} or Weir and Cockerham's (1984) Θ gives a measure of the amount of genetic variation among populations that is due to interpopulational genetic differences. Values for this index range from 0 to 1 indicating zero to complete population subdivision.

Haplotype Frequency Analysis

Statistical significance of haplotype frequency differences between sampling sites within the major geographic sampling areas was tested using a randomized generation of the x^2 distribution by the Monte Carlo procedure (Roff and Bentzen

1989). Two populations were considered to have significant differences if higher x^2 values were generated in 5% or less of 1000 randomized generations. This analysis is very conservative, and was designed for use with the finite sample sizes typical of most molecular analyses. In this study, randomizations were performed by the program MONTE (R.G. Danzman, University of Guelph, Guelph, Ontario, Canada).

Log likelihood ratio x^2 or G-tests were used to test for differences in haplotype frequency between populations. Sampling locations within these regions were combined for the analysis.

RESULTS

Gender Determination

Amplification with the ZFY/ZFX primer set (Palsbøll *et al.* 1992) and subsequent digestion with *Taq*I gave distinct patterns for male and female belugas (Fig. 1.1). Females were identified by a single band of size slightly less than 0.5 kb, while males yielded an additional band a little over 0.6 kb in size. This reflects differential patterns of restriction sites on the X and Y chromosomes. The Y chromosome has one *Taq*I site, dividing the amplified product into two fragments of size approximately 439 and 621 bp, while X chromosomes have an extra site within the larger product, cutting it into fragments of 439 and 182 bp. The 182 bp product runs off the end of the gel, thus leaving a single band consisting of the two 439 bp X chromosome fragments (Palsbøll *et al.* 1992). This information provided the sex of the samples collected.

Amplification and Sequencing

The sequence and location of primers used in this study are given in Table 1.1 and Figure 1.2. Amplification primers L15926 and H00651 (Kocher *et al.* 1989) targetted regions flanking the control region of beluga mtDNA, in the $tRNA^{phe}$ and 5-end of 12S RNA, respectively. Amplification products using this primer pair are approximately 1.1 kb long and span part of the $tRNA^{thr}$ gene, the

FIGURE 1.1. Restriction enzyme patterns for male (♂) and female (♀) belugas of DNA amplified using primers targetting the X and Y chromosomes and digested with *Taq*I. The sizes, in Kb, of the marker fragments of similar size range, a portion of a 100 bp size ladder (Bethesda Research Laboratories), are indicated on the left side.

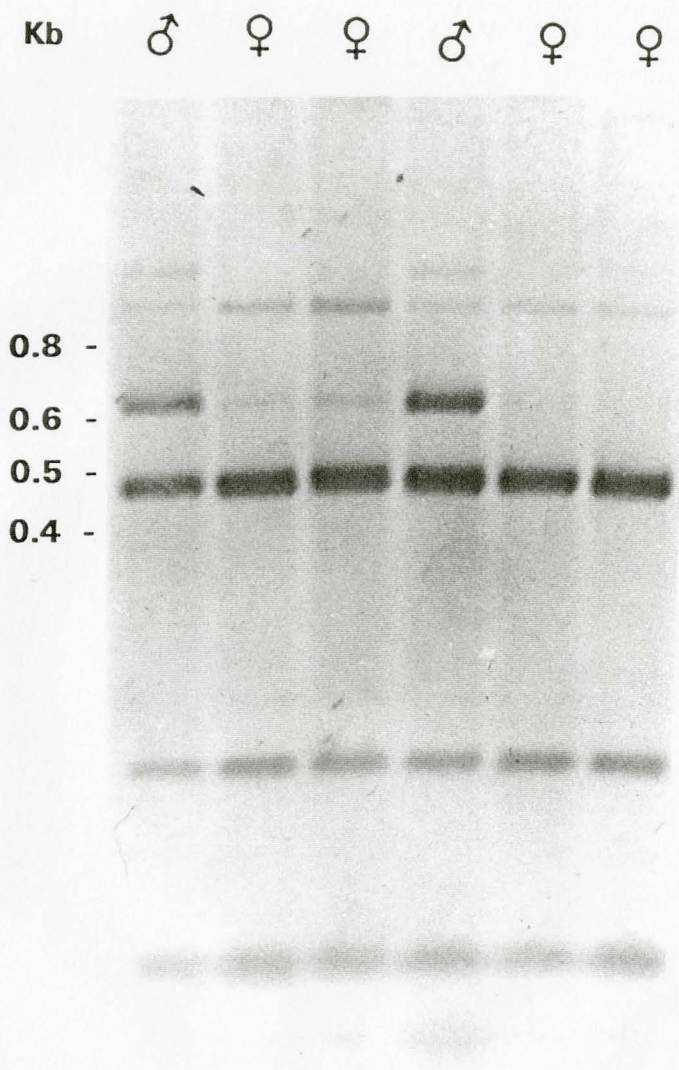
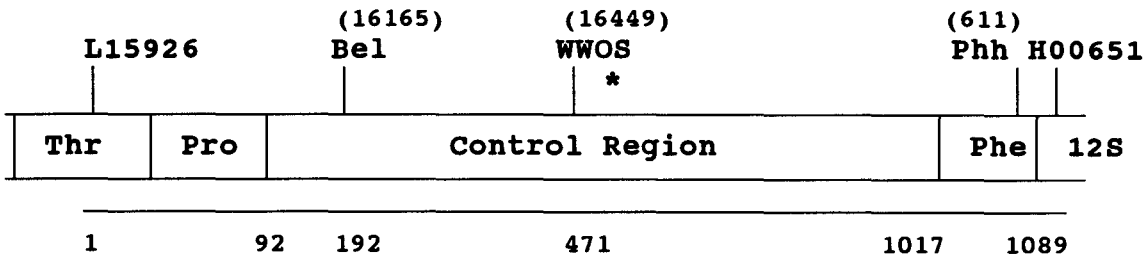

Kb
♂
♀
♀
♂
♀
♀
0.8 -
0.6 -
0.5 -
0.4 -

TABLE 1.1. Primers used in amplification and sequencing of the beluga control region.

Primer	Sequence
Amplification	
L15296[1]	5′-TCAAAGCTTACACCAGTCTTGTAAACC-3′
H00651[1]	5′-TAACTGCAGAAGGCTAGGACCAAACCT-3′
Phh[2]	5′-CCATCTAGACATTTTCAGTG-3′
Sequencing	
Bel 5′[2]	5′-ACATTTTACTGTGACTATTG-3′
WWOS	5′-GAGATATGGGCCCGGTGCGAG-3′

[1] from Kocher *et al.* (1989).
[2] R.W. Lillie, unpublished.
[3] Numbers refer to the position of the 3′ end of the oligonucleotide with reference to the human mtDNA sequence (Anderson *et al.* 1981).

FIG. 1.2. Schematic of approximate primer locations on the mtDNA molecule.



[a]Thr,Pro, Phe, and 12S refer to threonine, proline and phenylalanine transfer RNA genes, and 12S RNA gene, respectively. Numbers in parentheses indicate approximate locations of primers corresponding to the human sequence (Anderson *et al.* 1981). Below the diagram is a scale showing distances along the amplified product.
* indicates the location where numbering of the human mtDNA begins.

$tRNA^{pro}$ gene, the control region, and the $tRNA^{phe}$ gene (cf. Southern *et al.* 1988). Reamplification utilized the nested Phh primer designed by R. Lillie (pers. comm.), which targets the heavy strand in the $tRNA^{phe}$, in combination with L15926 to yield a single 1.05 kb product (Fig. 1.3).

A total of 320 bp were sequenced in the control region, encompassing base positions 91 to 410 of the light strand of beluga mtDNA (R. Lillie, unpublished data). This segment encompasses 213 bp of the highly variable 5' end and 107 bp of the conserved central region (cf. Anderson *et al.* 1982, Southern *et al.* 1988). The heavy strand was sequenced, for proofreading purposes, over the region between positions 371 to 91 (L-strand). The control region was therefore analyzed using 281 bp sequenced from both strands. The additional 39 bp comprises the region over which clear light-strand sequences were generated in all samples.

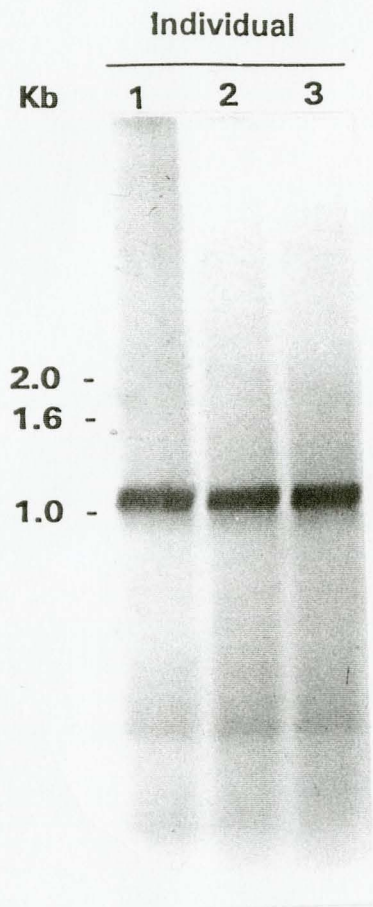
Sequence Variation

Mitochondrial DNA from 126 belugas from 4 geographic regions and 12 sampling sites on the Nunavik coast were analysed (Table 1.2 and Fig. 1.4). These included samples collected at summering areas as well as at Wakeham Bay during autumn occupation. However, 5 animals identified as possible calves belonging to females also included in analyses were removed from the study.

Seventeen variable positions, all transition substitutions (A↔G/C↔T), were identified. From these, 17 haplotypes were identified in belugas as sequences differing at one or more sites (Table 1.3). Most of the variation (12 sites) occurred within the 213 bp sequenced from the 5' variable region of the D-loop. Alignment with harbour porpoise (*Phocoena phocoena*, Rosel 1992) and killer

FIGURE 1.3. Products of (a) amplification and (b) reamplification of mitochondrial DNA control region. Numbers along the left sides of gels indicate marker fragments of similar range to the PCR products, and are portions of (a) the 1 kb size marker and (b) the 100 bp size marker (Bethesda Research Laboratories). Each initial amplification product was used for two reamplifications which were then pooled for purification. Individual #1 is an example of insufficient reamplification product.

(a)



(b)

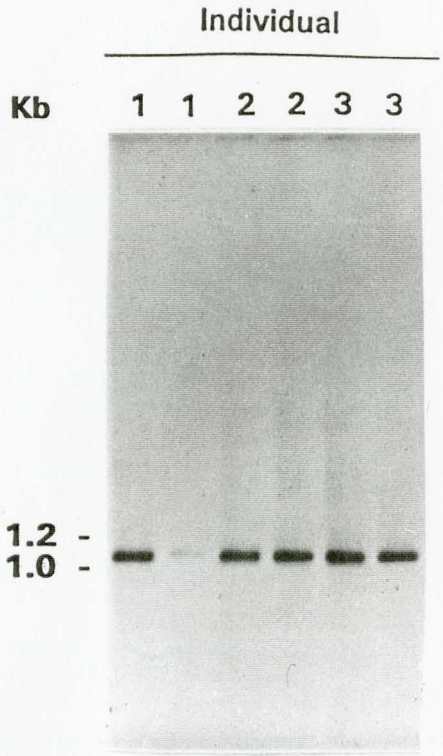


TABLE 1.2. Numbers of beluga samples from each sampling location.

	♀	♂	Total
Summer Samples			
Nastapoka River	29	19 (2)[1]	48 (2)
Eastmain			
Little Whale River/ Second River	5 (1)	3 (1)	8 (2)
Great Whale	7	2	9
TOTALS	12 (1)	5 (1)	17 (2)
Southern Hudson Strait			
Salluit	3	4	7
Kangiqsujjuaq	0	1	1
Quagtuq	6	7	13
TOTALS	9	12	21
Ungava Bay			
Kangirsuk	4	2	6
Kujjuaq	1	1	2
George River	0	1	1
Tasiujaq	2	0	2
TOTALS	7	4	11
Winter Samples			
Wakeham Bay	9	15	24

[1]Numbers in parentheses indicate samples removed from analysis as possible calves of female samples.

FIGURE 1.4. Map of Nunavik showing sampling sites and place names mentioned in the text. (Modified from Finley *et al.* 1982).

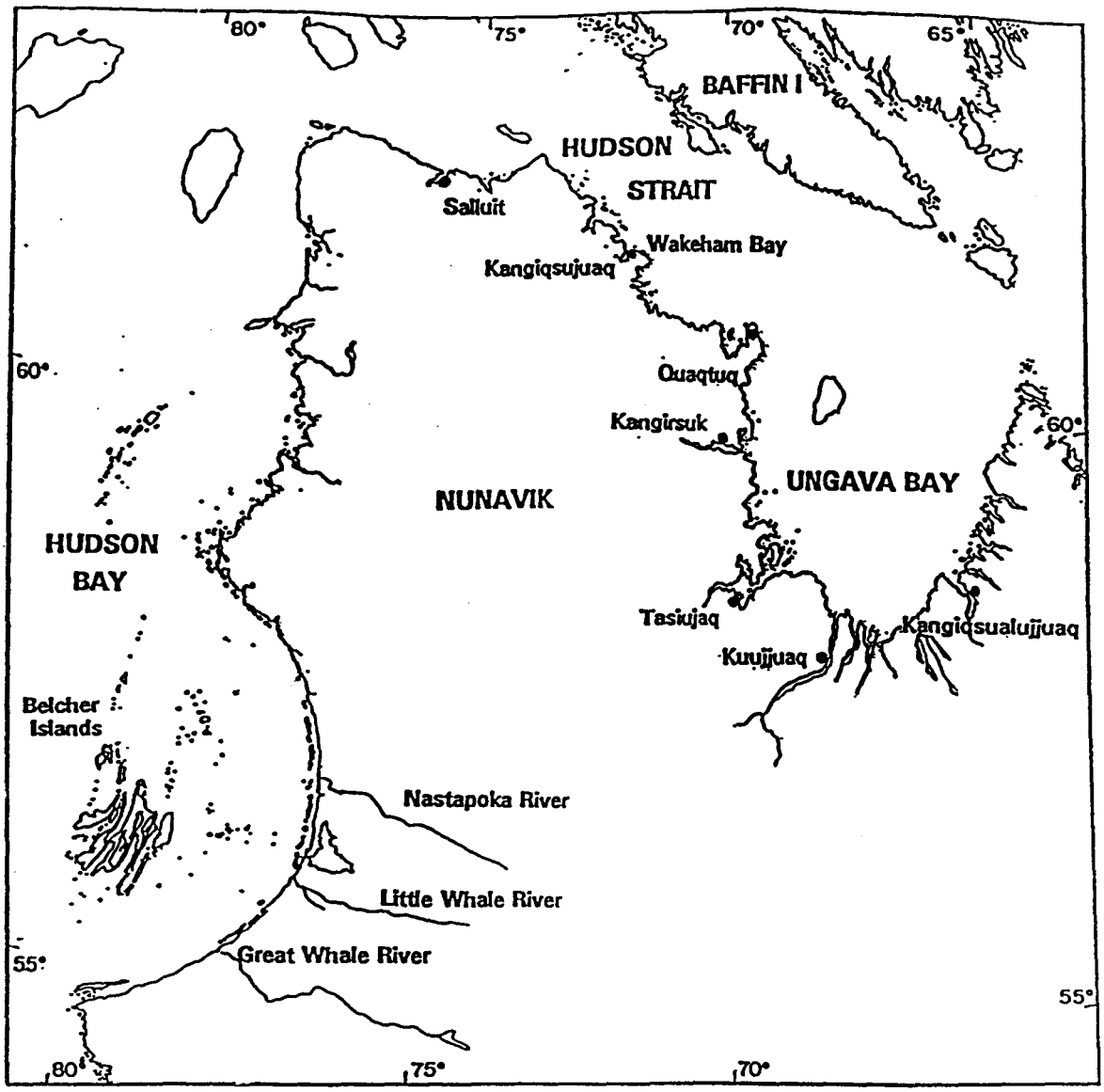

80°
75°
70°
65°
BAFFIN I
HUDSON
STRAIT
Salluit
Wakeham Bay
Kangiqsujuaq
60°
Quaqtuq
Kangirsuk
60°
UNGAVA BAY
NUNAVIK
HUDSON
BAY
Tasiujaq
Kangiqsualujjuaq
Kuujjuaq
Belcher
Islands
Nastapoka River
Little Whale River
55°
Great Whale River
55°
80°
75°
70°

TABLE 1.3. Variable nucleotide positions in 320 bp in 17 haplotypes found in 126 belugas.

Haplo	97	149	166	191	192	211	232	267	272	274	280	281	325	389	391	407	408
D01	T	C	T	A	C	A	T	G	C	A	C	C	C	T	C	T	G
D02	•	•	•	•	•	•	C	•	•	•	•	•	•	•	T	•	A
D03	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•
D04	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A
D05	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	A
D06	C	T	•	•	T	G	C	A	•	•	•	T	T	•	T	•	A
D07	C	T	•	G	T	G	C	A	•	•	T	•	T	•	T	•	A
D08	C	T	•	G	T	G	C	A	•	•	•	•	T	•	T	•	A
D09	C	T	•	G	T	G	C	A	•	•	•	T	T	•	T	C	A
D10	C	T	•	G	T	G	C	A	•	•	•	•	T	•	T	C	A
D11	C	T	•	G	T	•	C	A	•	•	•	•	T	•	T	•	A
D12	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	A
D13	C	T	•	G	T	G	C	A	•	•	•	•	•	•	T	•	A
D14	C	T	•	G	T	G	C	•	•	•	•	•	T	•	T	•	A
D15	•	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	A
D16	C	T	•	G	T	G	C	A	•	G	•	•	T	•	T	•	A
D17	C	T	C	•	T	G	C	A	•	•	•	T	T	•	T	•	A

Numbers indicate position of site in the beluga control region (R.W. Lillie, unpublished). Nucleotides identical to those in haplotype D01 are denoted by •. The range of positions shown here corresponds to positions 16,170 to 16,483 of the human mitochondrial DNA sequence.

whale (*Orcinus orca*, Hoelzel *et al.* 1991) sequences reveals high conservation within the central region (Fig. 1.5), as well as in conserved blocks *H* (91%) and *a* (94%) described by Anderson *et al.* (1982) and Dillon and Wright (1993). Conserved blocks *I* and *J* showed less conservation (77% and 81%), though more than reported by Southern *et al.* (1988) for dolphin, where these blocks are not found.

Phylogenetic Analyses

Phylogenetic analyses were performed to determine the genetic relationships between haplotypes. Harbor porpoise (*Phocoena phocoena*) was used as an outgroup due to the placement of Phocoenidae in phylogenetic analyses as closely related to Monodontidae in relation to other cetacean families (e.g. Milinkovitch *et al.* 1994). Killer whale (*Orcinus orca*) sequences were used because of availability of a complete sequence in the literature (Hoelzel *et al.* 1991). Neighbour-joining trees constructed using PHYLIP show the division of beluga control region haplotypes into two clades, referred to as Groups A and B, which appear similar to RFLP linages detected by Brennin (1992; Brennin *et al.*, in prep.). A strict consensus tree assembled from 500 bootstrap replicates of the NJ tree supported this division (Fig. 1.6). Maximum-likelihood analysis gave similar results. Pairwise sequence divergence between A and B group haplotypes ranged from 1.9% to 4.1%, much higher than within either clade (0.3% - 0.9% within A; 0.3% - 1.3% within B) (Table 1.4).

FIGURE 1.5. Representative control region sequence of the beluga whale (DLE) aligned with that of the killer whale (OOR) and harbor porpoise (PPH). The beluga sequence presented here corresponds to haplotype D01. Variable positions identified in belugas in this study are in underlined lower case. Bases identical to those in the beluga sequence are represented by dots. Dashes indicate insertions or deletions. Letters *E-K* and *a* indicate conserved sequence blocks as defined by Anderson et al. (1982), Southern *et al.* (1988), and Dillon and Wright (1990).

→ denotes the beginning of the central region.

* denotes the position of the end of the WWOS heavy-strand sequence primer.

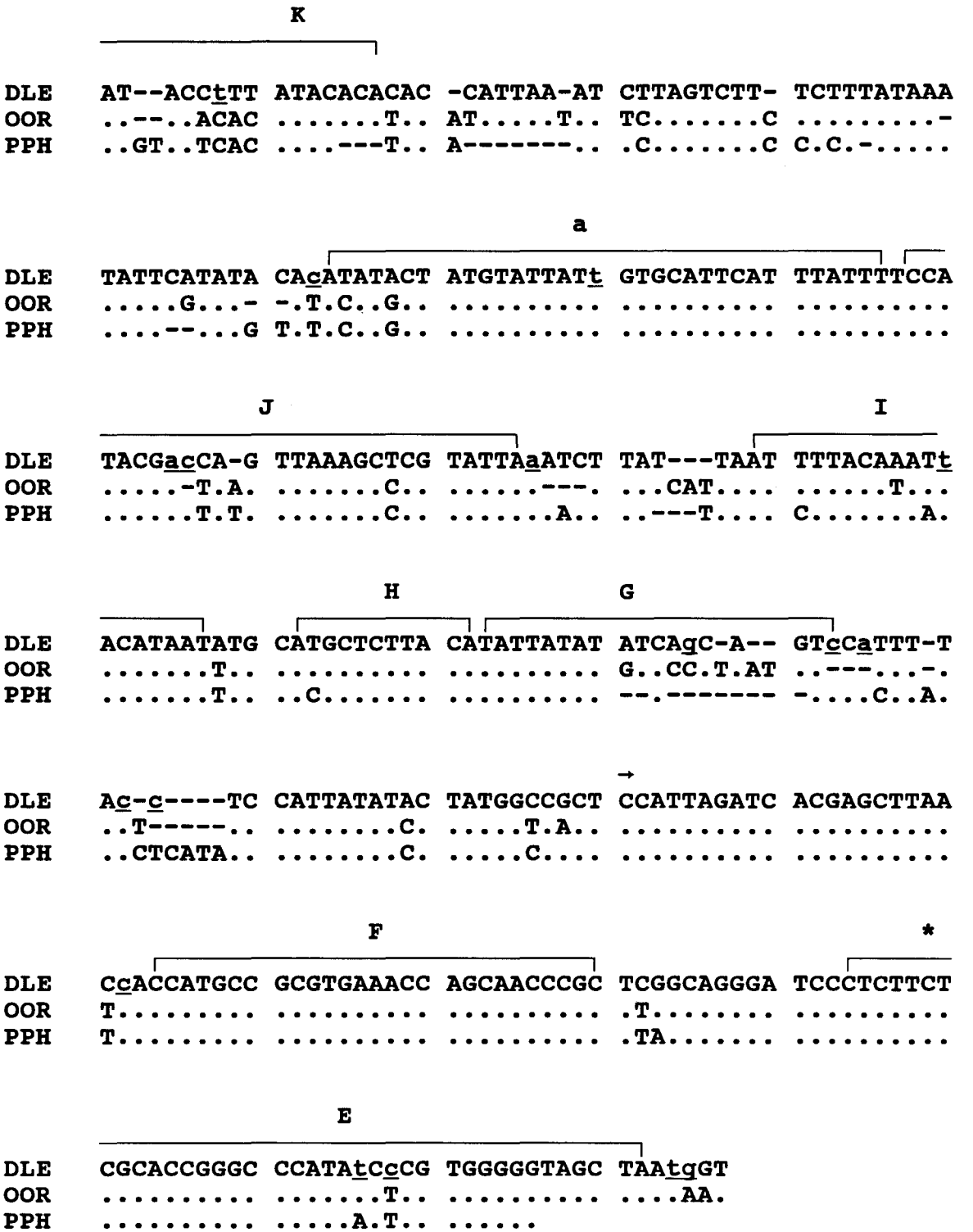

K
DLE AT--ACCtTT ATACACACAC -CATTAA-AT CTTAGTCTT- TCTTTATAAA
OOR ..--..ACAC ....T.. AT.....T.. TC.......C ....-
PPH ..GT..TCAC ....---T.. A-------.. .C.......C C.C.-.....
a
DLE TATTCATATA CAcATATACT ATGTATTATt GTGCATTCAT TTATTTTCCA
OOR .....G...- -.T.C..G.. ....
PPH ....--...G T.T.C..G.. ....
J
I
DLE TACGacCA-G TTAAAGCTCG TATTAaATCT TAT---TAAT TTTACAAATt
OOR .....-T.A. ....C.. ....---. ...CAT.... ......T...
PPH ......T.T. ....C.. ....A.. ..---T.... C.......A.
H
G
DLE ACATAATATG CATGCTCTTA CATATTATAT ATCAgC-A-- GTcCaTTT-T
OOR ....T.. .... G..CC.T.AT ..---...-.
PPH ....T.. ..C....... .... --.------- -....C..A.
→
DLE Ac-c----TC CATTATATAC TATGGCCGCT CCATTAGATC ACGAGCTTAA
OOR ..T-----.. ....C. .....T.A.. ....
PPH ..CTCATA.. ....C. .....C.... ....
F
*
DLE CcACCATGCC GCGTGAAACC AGCAACCCGC TCGGCAGGGA TCCCTCTTCT
OOR T......... .... .T......... ....
PPH T......... .... .TA........ ....
E
DLE CGCACCGGGC CCATAtCcCG TGGGGGTAGC TAAtgGT
OOR ....T.. ....AA.
PPH ....A.T.. ......

FIGURE 1.6. Phylogenetic relationships among control region sequence haplotypes of belugas sampled in Nunavik waters. Numbers on branches indicate percentage of 500 bootstrap replicates showing consensus with initial neighbor-joining analysis. The outgroups included are killer whale (OOR) and harbour porpoise (PPH).

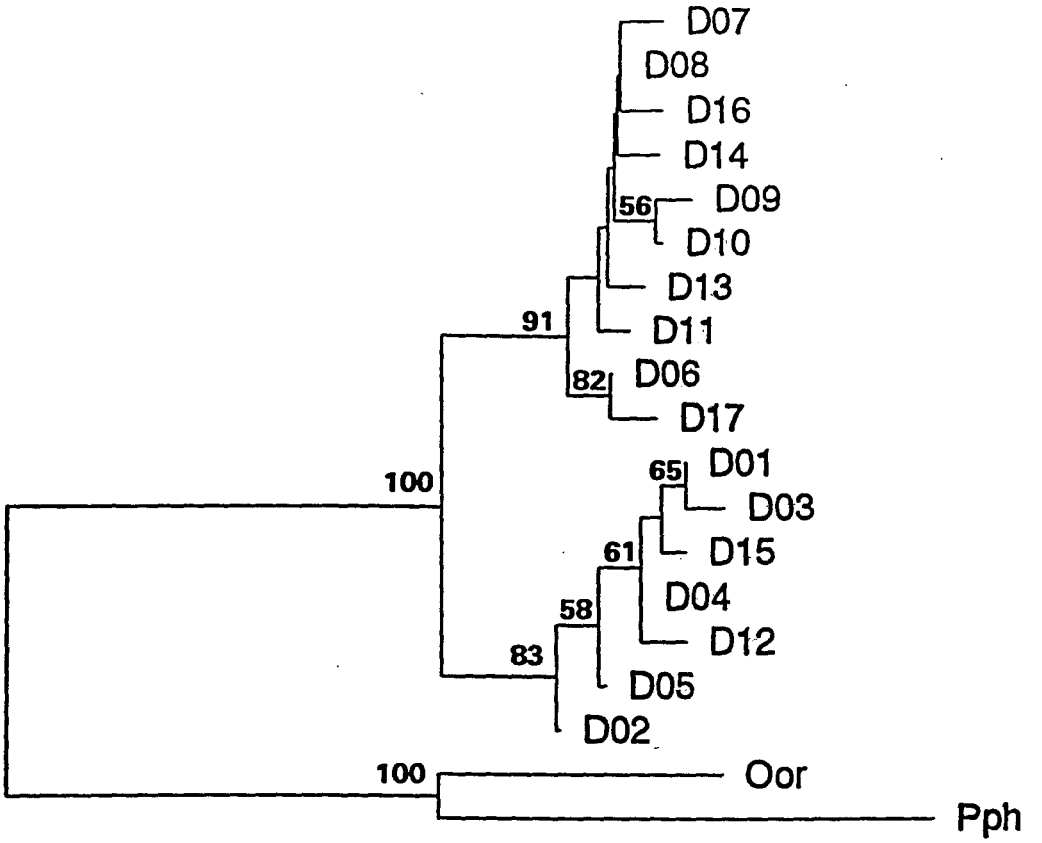

D07
D08
D16
D14
56
D09
D10
D13
91
D11
82
D06
D17
100
65
D01
D03
61
D15
58
D04
83
D12
D05
D02
100
Oor
Pph

TABLE 1.4. Percent sequence divergence between beluga haplotypes. Values above the diagonal show the number of substitutions occurring between haplotypes. Values below diagonal are the pairwise sequence differences expressed in percent (%).

	D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12	D13	D14	D15	D16	D17
D01	-	3	1	1	2	10	11	10	12	11	9	2	9	9	2	11	11
D02	0.9	-	3	2	1	7	8	7	9	8	6	3	6	6	3	8	8
D03	0.3	0.9	-	2	3	11	12	11	13	12	10	3	10	10	1	12	12
D04	0.3	0.6	0.6	-	1	9	10	9	11	10	11	1	8	8	1	10	10
D05	0.6	0.3	0.9	0.3	-	8	9	8	10	9	7	2	7	7	2	9	9
D06	3.1	2.2	3.5	2.8	2.5	-	3	2	2	3	3	10	3	3	10	3	1
D07	3.5	2.5	3.8	3.1	2.8	0.9	-	1	3	2	2	11	2	2	11	2	4
D08	3.1	2.2	3.5	2.8	2.5	0.6	0.3	-	2	1	1	10	1	1	10	1	3
D09	3.8	2.8	4.1	3.5	3.1	0.6	0.9	0.6	-	1	3	12	3	3	12	3	3
D10	3.5	2.5	3.8	3.1	2.8	0.9	0.6	0.3	0.3	-	2	11	2	2	11	2	4
D11	2.8	1.9	3.1	3.5	2.2	0.9	0.6	0.3	0.9	0.6	-	9	2	2	9	2	4
D12	0.6	0.9	0.9	0.3	0.6	3.1	3.5	3.1	3.8	3.5	2.8	-	9	9	2	11	11
D13	2.8	1.9	3.1	2.5	2.2	0.9	0.6	0.3	0.9	0.6	0.6	2.8	-	2	9	2	4
D14	2.8	1.9	3.1	2.5	2.2	0.9	0.6	0.3	0.9	0.6	0.6	2.8	0.6	-	9	9	4
D15	0.6	0.9	0.3	0.3	0.6	3.1	3.5	3.1	3.8	3.5	2.8	0.6	2.8	2.8	-	11	11
D16	3.5	2.5	3.8	3.1	2.8	0.9	0.6	0.3	0.9	0.6	0.6	3.5	0.6	0.6	3.5	-	4
D17	3.5	2.5	3.8	3.1	2.8	0.3	1.3	0.9	0.9	1.3	1.3	3.5	1.3	1.3	3.5	1.3	-

Distribution of Haplotypes

Distributions and numbers of haplotypes are shown in Table 1.5 and Figure 1.7. Phylogenetically different haplotypes did not segregate geographically, as types from both groups were found to occur in all geographic areas sampled. However, each region surveyed was observed to be mostly composed of only one group. Randomized generations of x^2 distributions were used to assess the significance of different haplotype frequencies between sampling sites within a given geographic region (Table 1.6). Frequency differences were not found to be significant in any comparisons except between Nastapoka and Great Whale (x^2 = 10.94, $p < 0.009$) and Nastapoka and Little Whale ($x^2 = 3.18$, $p = 0.011$).

G-tests of independence (Table 1.7) found significant differences in haplotype frequencies in comparisons of eastern Hudson Bay to both southern Hudson Strait ($G = 25.36$, $p << 0.001$) and Ungava Bay ($G = 20.94$, $p << 0.001$), indicating genetically distinct populations. Significant differences were found between Nastapoka and Little Whale/Great Whale allele frequencies ($G = 5.74$, $p < 0.025$). Southern Hudson Strait did not differ significantly from Ungava Bay ($G = 0.56$, $p > 0.25$).

Certain haplotypes were found to be predominant within a given region. Two A group haplotypes, D01 and D04, were found in 60% of animals from the eastern Hudson Bay, and 75% from the Nastapoka River alone. D01 was present in only 2 animals from the northern regions (Ungava Bay and Southern Hudson Strait), while D04 was restricted to Hudson Bay summering areas. Alternatively, haplotype D08 (Group B) accounted for 53% of sequences from the two northern regions, and 68% of Southern Hudson Strait, but was found in only 3 of 65

TABLE 1.5. Numbers of haplotype groups A and B and numbers of each haplotype at each sampling location.

	A	B	Haplotype counts
Summer Samples			
Nastapoka R.	42	6	A- D01-23, D02-1, D03-4, D04-13,D05-1 B- D06-3, D07-2, D08-1
Eastmain			
Little Whale R./ Second R.	5	3	A- D02-2, D05-2, D12-1 B- D08-2, D09-1
Great Whale R.	5	4	A- D01-1, D02-1, D04-1 D05-2 B- D06-2, D16-1, D17-1
TOTALS	10	7	
Southern Hudson Strait			
Salluit	0	7	B- D08-6, D11-1
Kangiqsujuaq	0	1	B- D08-1
Quaqtuq	4	9	A- D01-1, D02-1, D05-2 B- D06-2, D08-6, D10-1
TOTALS	4	17	
Ungava Bay			
Kangirsuk	1	5	A- D01-1 B- D06-2, D08-2, D11-1
Kujjuaq	0	2	B- D08-1, D10-1
Kanqiqsualujjuaq	0	1	B- D13-1
Tasiujaq	0	2	B- D08-1, D11-1
TOTALS	1	10	
Autumn Samples			
Wakeham Bay	9	15	A- D02-2, D03-1, D04-1 D05-2, D15-3 B- D06-1, D08-10, D09-2, D13-1, D14-1

FIGURE 1.7. Geographic distribution of haplotype groups A (black) and B (white). Sample sizes are shown in parentheses.

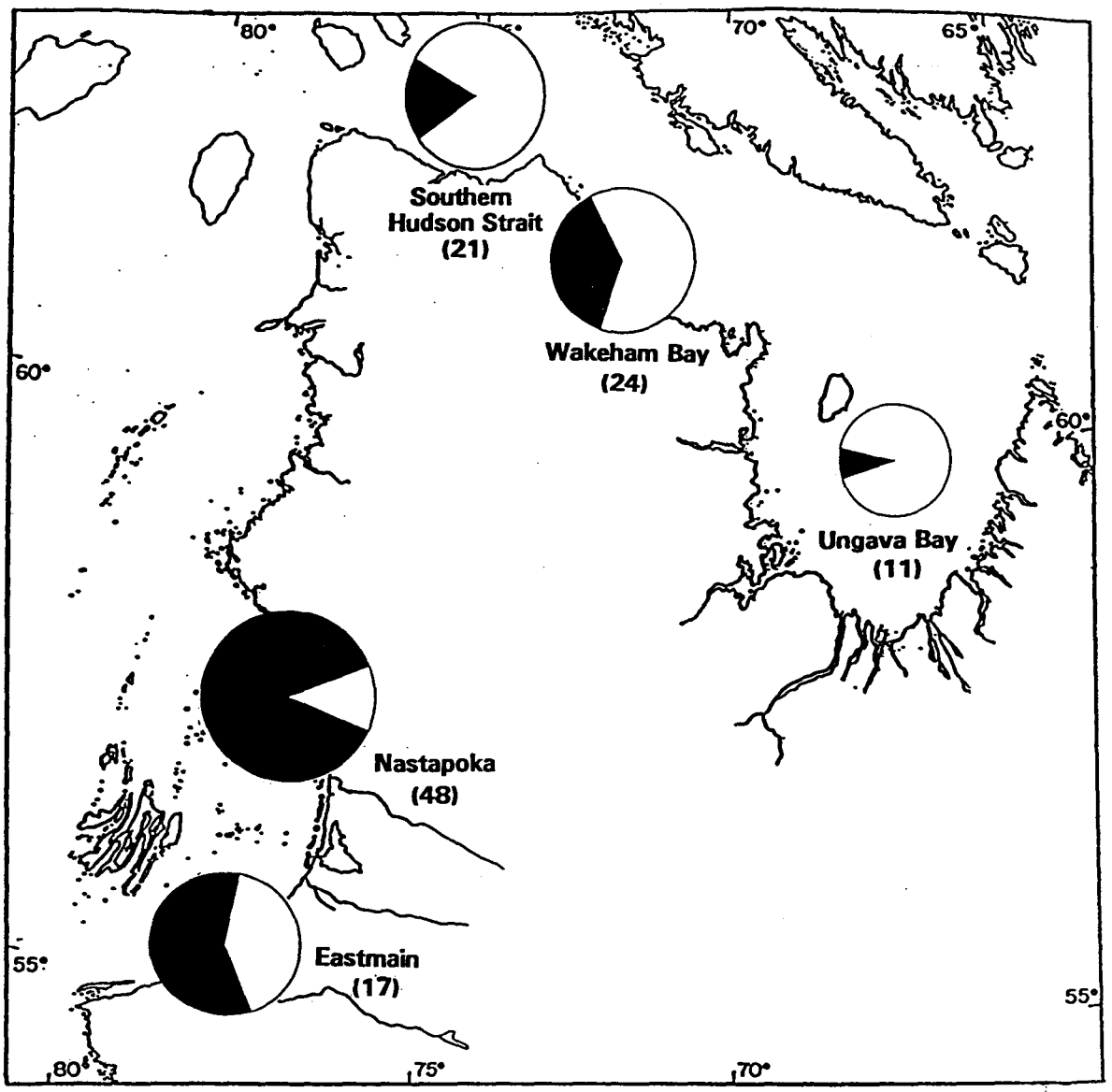

80°
70°
65°
Southern
Hudson Strait
(21)
Wakeham Bay
(24)
60°
60°
Ungava Bay
(11)
Nastapoka
(48)
Eastmain
(17)
55°
55°
80°
75°
70°

TABLE 1.6. Analysis of the frequency distribution of haplotype groups A and B among sample sites within provisional subpopulations of belugas in Nunavik waters.

	p		p
Eastern Hudson Bay		**Southern Hudson Strait**	
NR-LWR[1]	0.011*	SAL/KAN-QUAQ	0.010*
NR-GWR	0.009*	SAL-QUAQ	0.099
LR-GWR	0.090		
		Ungava Bay	
		KANG-SUB[2]	0.463

Values presented are the adjusted probabilities of 1000 Monte Carlo bootstrapping randomizations using the method of Roff and Bentzen (1989).

[1]Nastapoka (NR), Little Whale(LWR), Great Whale (GWR), Salluit (SAL), Kangiqsujjuag (KAN), Quaqtuq (QUAQ), Kangirsuk (KANG), Southern Ungava Bay (SUB).

[2]Southern Ungava Bay is a pooled data set including Kujjuaq, George River, and Tasiujaq.

* indicates significant differences between populations.

TABLE 1.7. Analysis of the frequency distribution of haplotype groups A and B among provisional populations in Nunavik waters.

Single population comparisons		
NR-EAS[1]	5.74	($p < 0.025$)
NR-SHS	30.67	($p << 0.001$)
NR-UB	25.40	($p << 0.001$)
EAS-SHS	6.36	($p < 0.25$)
EAS-UB	7.50	($p < 0.001$)
SHS-UB	0.56	($p > 0.25$)
Combined population comparisons		
NR/EAS-SHS	25.36	($p << 0.001$)
NR/EAS-UB	20.94	($p << 0.001$)
NR-SHS/UB	43.97	($p << 0.001$)
EAS-SHS/UB	9.37	($p < 0.005$)
NR/EAS-SHS/UB	38.24	($p << 0.001$)

Values presented are results of G-tests of independence with Williams' correction (Sokal and Rohlf 1987). Associated probabilities (d.f. = 1) are in parentheses.

[1]Nastapoka (NR), Eastmain (EAS), Southern Hudson Strait (SHS), Ungava Bay (UB). Eastmain (EAS) is a pooled data set including Little Whale and Great Whale.

Hudson Bay samples. This haplotype was also predominant among autumn samples.

There was a more mixed distribution of Group A and B haplotypes in the samples collected at Wakeham Bay, indicating intermixing of summering populations on overwintering grounds. However, G-tests (Table 1.8) showed allele frequencies were not significantly different than those observed in Southern Hudson Strait ($G = 1.85$, $p < 0.25$) or Ungava Bay ($G = 3.3$, $p < 0.10$), although the randomized x^2 test did indicate Ungava Bay and Wakeham Bay are different ($x^2 = 2.98$, $p = 0.05$). The presence of several A and B haplotypes nonetheless suggests a genetic composition of wintering belugas consisting of animals from both northern and eastern Nunavik waters.

Intra- and Interpopulation Indices

Estimates of haplotype and nucleotide diversities within regions are given in Table 1.9. Measures of haplotype diversity (h) ranged from 0.62 (Southern Hudson Strait, $N = 21$) to 0.92 (Eastmain, $N = 17$). Estimates taken for the pooled northern and southern populations were similar: 0.79 for eastern Hudson Bay ($N = 68$), 0.70 for Hudson Strait/Ungava Bay ($N = 32$), and 0.81 among autumn Wakeham samples ($N = 24$).

Nucleotide diversity (π) was high in Eastmain (1.69%) and Wakeham Bay (1.61%) compared to average values for mtDNA of marine species ($\approx$ 1%; Ovenden 1992). Values for other areas ranged from .88% (Ungava Bay) to 1.01% (Hudson Strait). π-values for pooled northern and southern summering areas gave .97% and 1.75%, respectively.

TABLE 1.8. Analysis of differences in the frequency distribution of haplotype groups A and B between samples taken at Wakeham Bay in autumn and at summering populations.

Single population comparisons		
NR-WB[1]	18.68	($p << 0.001$)
WB-EAS[2]	1.79	($p < 0.25$)
WB-SHS	1.85	($p < 0.25$)
WB-UB	3.30	($p << 0.10$)
Combined population comparisons		
WB-NR/EAS	13.84	($p < 0.01$)
WB-SHS/UB	3.40	($p < 0.10$)
WB-SUMMER SAMPLES	27.93	($p << 0.001$)

Values presented are results of G-tests of independence with Williams' correction (Sokal and Rohlf 1987). Associated probabilities (d.f. = 1) are in parentheses.

[1]Nastapoka (NR), Eastmain (EAS), Southern Hudson Strait (SHS), Ungava Bay (UB).

[2]Eastmain (EAS) is a pooled data set including Little Whale and Great Whale.

TABLE 1.9. Diversity indices of beluga whale mitochondrial DNA within provisional subpopulations in Nunavik waters.

Provisional Subpopulations	Haplotypic Diversity	Nucleotide Diversity (x100)
Nastapoka	0.70	0.919
Eastmain[1]	0.92	1.689
Southern Hudson Strait	0.62	1.010
Ungava Bay	0.85	0.885
Nastapoka/Eastmain	0.79	1.268
Southern Hudson Strait/Ungava Bay	0.70	0.970
Wakeham Bay	0.81	1.606

Haplotypic diversity was estimated using equation 7 of Nei and Tajima (1981). Nucleotide diversity was estimated using equation 10.6 in Nei (1987).

[1]Eastmain is a combined data set including Little Whale and Great Whale.

Genetic divergence, both uncorrected (d_{XY}) and corrected for intrapopulation diversity (d_A) are given in Table 1.10. Estimates between Hudson Bay and the northern groups clustered around 1.1%, indicating substantial isolation of populations. Comparisons within Hudson Bay (Nastapoka-LW/GW) were lower (0.40%), suggesting some degree of exchange. The divergence between Hudson Strait and Ungava Bay gave a negative value, indicating no substantial divergence between populations.

N_{ST} (Lynch and Crease 1990) values between areas in eastern Hudson Bay and South Hudson Strait/Ungava Bay ranged from 0.19 - 0.22 for comparisons involving LW/GW, to 0.60 - 0.63 for those involving Nastapoka (Table 1.11). N_{ST} between eastern Hudson Bay and the northern localities was 0.51. This indicates that 51% of the variation found in summering areas could be attributed to differences between the two major geographic areas. This is consistent with a view of genetically distinct beluga stocks in eastern Hudson Bay and southern Hudson Strait/Ungava Bay.

TABLE 1.10. Nucleotide divergence between putative subpopulations of beluga whales in Nunavik waters. Above and below the diagonal are the values (x100) corrected and uncorrected for intrapopulation diversity, respectively.

	NR	EAS	SHS	UB	NR/EAS	SHS/UB	WB
NR[1]	-	0.404	1.446	1.571	n.a.	1.524	0.819
EAS	1.708	-	0.331	0.396	n.a.	0.378	0.516
SHS	2.411	1.681	-	-0.049	1.079	n.a.	0.047
UB	2.473	1.684	0.897	-	1.188	n.a.	0.081
NR/EAS	n.a.	n.a.	2.218	2.265	-	1.149	0.542
SHS/UB	2.469	1.707	n.a.	n.a.	2.268	-	0.075
WB	2.082	1.700	1.326	1.355	1.979	1.364	-

[1]Abbreviations: NR - Nastapoka River; EAS - Eastmain; SHS - Southern Hudson Strait; UB - Ungava Bay; WB - Wakeham Bay, fall samples.
Nucleotide divergence was calculated as outlined in Nei (1987).

TABLE 1.11. N_{ST} for various provisional populations of beluga whales.

Single populations		Combined populations	
NR-EAS[1]	0.206	NR-SHS/UB	0.617
NR-SHS	0.560	EAS-SHS/UB	0.221
NR-UB	0.635		
		NR/E-SHS	0.486
EAS-SHS	0.197	NR/E-UB	0.525
EAS-UB	0.236		
		NR/E-SHS/UB	0.507
SHS-UB	-0.055		

[1]Abbreviations: NR - Nastapoka River; EAS - Eastmain; SHS - Southern Hudson Strait; UB - Ungava Bay; WB - Wakeham Bay, fall samples.

DISCUSSION

Control Region Variability

The control region sequence of the beluga whale shows patterns of variation similar to other odontocete species (Southern *et al.* 1988; Dillon and Wright 1993). The portion of the central D-loop region sequenced for this study (approximately one-third) shows high sequence similarity among the beluga, harbor porpoise and killer whale sequences aligned here, both within and between similarity blocks E and F designated by Anderson *et al.* (1982). This region, covered by 7S DNA, the newly synthesized DNA strand involved in the displacement loop formed during replication of mtDNA, has been shown to be the most conservative region in D-loops of humans (Anderson *et al.* 1981), cow (Anderson *et al.* 1982), sheep (Upholt and Dawid 1977), and in several cetacean species (Southern *et al.* 1988; Dillon and Wright 1990), implying some functional importance of this region. The central region has been implicated in regulation of heavy strand replication (Clayton 1982), and an open reading frame of variable length among species has been reported in this area (Saccone *et al.* 1987; Hoelzel *et al.* 1991).

Patterns of variability within the 5' or left domain of the control region are also similar to those reported for other cetaceans. Similarity within conserved blocks *H* (Anderson *et al.* 1982; Southern *et al.* 1988) and *a* (Dillon and Wright

1990) was comparable to other species. However, conservation of blocks *I* and *J*, not found by those authors, was fairly high among the species compared here. This indicates that the 5' region is free to vary more than the adjacent central domain among cetaceans, as it is for other taxa (Saccone *et al.* 1987). Higher variability within the 5' end reported for other cetacean species (Hoelzel *et al.* 1991b; Baker *et al.* 1993) was seen among beluga sequences as well. Twelve of the seventeen variable sites identified in the beluga control sequences were located scattered among the 213 bp sequenced in the 5' end of the D-loop.

The seventeen haplotypes identified among the 126 beluga sequences differed from each other by 1 to 13 substitutions. This corresponds to sequence divergence estimates of 0.31% - 4.1% between the various haplotypes. This range is comparable to that found in similar surveys of intraspecific sequence variation in humpback whales (0.3% - 5.65%; Baker *et al.* 1993), ghost bat (0.3% - 3.5%; Wilmer *et al.* 1994), and sturgeon (0.01% - 4.29%; Brown *et al.* 1993). The absence of transversions among beluga sequences is consistent with a high transition to transversion bias observed in vertebrates (Brown and Simpson 1982; Brown *et al.* 1982; Aquadro and Greenburg 1983; Hixson and Brown 1986).

Haplotype Distributions

Bootstrap resampling strongly supported the division of haplotypes into two clades by neighbor-joining and maximium-likelihood analysis. This split is consistent with the splitting of mtDNA RFLP haplotypes into two lineages as shown by Brennin (1992; Brennin *et al.*, in prep.). In that study lineage I and II haplotypes were found mostly in populations derived from "eastern" and "western"

ancestral populations, respectively. Likewise, each sequence haplotype group was predominantly found in one or the other of the eastern Hudson Bay and northern Nunavik coast populations. The A and B groups found in this study likely correspond to the RFLP lineages I and II, respectively.

Within the eastern Hudson Bay samples, particularly from Nastapoka River, there was a predominance of Group A haplotypes. This may reflect a possible sampling bias, as collection within Nastapoka involved several days on which a number of kills occurred. Three mother-calf pairs were identified based on behavioural observations. It is possible that more of the animals of identical haplotype caught on the same day were first or second order relatives, in which case haplotype frequencies of these types could be inflated. Conversely, these could represent members of varying levels of relationship within a matriline. Such a possibility would support the learning of philopatry by calves as a basis for the structuring of mtDNA haplotypes over generations. RFLP analysis has been shown this to be the case for structuring of populations within Hudson Bay into eastern and western stocks (Brennin 1992; Brennin *et al.*, in prep.).

The prominence of two haplotypes (D01 and D04) which differ by only one position within Nastapoka samples suggests that pods which enter estuaries may be social groupings of highly related whales derived from one or two ancestral lineages. *Sampling within other estuaries in eastern Hudson Bay was scattered* over several dates, and show a mix of more variable haplotypes. Thus, it is not currently possible to determine whether the same situation occurs at other estuaries without a similar same-day collection.

Within the northern populations, B group haplotypes were more prominent.

These haplotypes were also identified in five animals sequenced from the Mackenzie Delta (data not shown), and similar to those seen in a parallel study involving several other beluga populations (J. Brown, personal communication). Haplotypes identical or highly similar to the B group types reported in this study have been found in the western Hudson Bay and Southeast Baffin populations. Southern Hudson Strait and Ungava Bay beluga populations may have been formed by animals recruited from either of these populations. Alternatively, they may represent independent colonization of Hudson Strait by "western" belugas after the Wisconsin Ice Age (see Brennin 1992).

Incomplete Concordance of Geography and Phylogeny

Phylogenetically different haplotype clades did not, however, completely segregate geographically. While each population was represented predominantly by one group or the other, there were present in each of them members of the other group. Thus, relatively divergent haplotypes occurred in close proximity to each other, and closely related sequences occurred across the sampled range. RFLP data showed a similar pattern of opposite-lineage haplotypes in eastern and western Hudson Bay populations (Brennin 1992; Brennin *et al.*, in prep.). Lack of full concordance between phylogeny and geography has also been observed in studies of the genetic population structure of various species (Cronin 1991, 1992; Plante *et al.* 1989a,b; Wayne *et al.* 1990; Avise *et al.* 1987; Ball *et al.* 1988).

An explanation for this type of pattern is secondary contact or gene flow between isolated populations (Avise *et al.* 1987; Taberlet *et al.* 1992). Exchange of females between populations resulting in the spread of mtDNA haplotypes

across geographic distances has been shown for animals with high mobility and dispersal such as canids (Wayne *et al.* 1990), cervids (Cronin 1991b), and bears (Cronin *et al.* 1991a).

Belugas from eastern Hudson Bay migrate hundreds of kilometres in autumn and spring, travelling to and from wintering grounds to the northeast of Hudson Bay, which they are believed to share with belugas from western Hudson Bay, Baffin Island and Ungava Bay populations (Finley *et al.* 1982; Richard *et al.* 1990). Recruitment of "foreign" haplotypes could occur by chance associations with females of a different population, or by premature cessation of migration at a different estuary due to ice conditions or calving. The presence of extra-group haplotypes in all populations indicates that some exchange has occurred in the past. It is not possible to determine whether this exchange occurred recently, or whether it reflects historical gene flow between populations, as haplotypes can remain within a population for a long period of time (Gyllenstein and Erlich 1989; Wayne *et al.* 1991).

Diversity

Calculated haplotype and nucleotide diversity indices for most beluga populations were comparable to other mammals (Wilson *et al.* 1985; Plante *et al.* 1989a; Wayne *et al.* 1990; O'Brien *et al.* 1990; Baker *et al.* 1993). Both indices were considerably high (5x - 7x) compared to those found within beluga populations using RFLP data (Brennin 1992; Brennin *et al.*, in prep.). This is to be expected because sequence analysis should uncover polymorphism not seen in analysis of restriction fragments. Several studies have shown greater levels of

diversity with sequence level data (e.g. Wayne *et al.* 1990; Brown *et al.* 1993; Lamb *et al.* 1994).

Nucleotide diversity within eastern Hudson Bay (1.69% for Eastmain, 1.27% Eastmain and Nastapoka) was particularly high, compared to that for most marine species ($\approx$ 1%, Ovenden 1992). Baker *et al.* (1993) reported values of 0.75-0.95% for humpback whales in Northern Pacific waters. The high nucleotide diversity in the eastern Hudson Bay populations reflects the presence of several animals with B-group haplotypes. Possible reasons for this will be explained later in this chapter.

Equally high (1.61%) nucleotide diversity was found in samples collected at Wakeham Bay in autumn. This is clearly reflective of the mixed-group nature of haplotype composition in the sample, which is consistent with a winter population composed of eastern and western Hudson Bay, Ungava Bay, Southern Hudson Strait, and possibly Southeast Baffin belugas. A mixture of haplotype groups or lineages was also found with RFLP data (Brennin 1992; Brennin *et al.*, in prep.). These combined results strongly support occupation of Hudson Strait in winter by animals from genetically distinct summering populations.

Diversity within Ungava Bay was higher than expected based on the size of the population. Surveys of this population reveal numbers too low to derive an estimate (e.g. $<$ 50; Smith and Hammill 1986). Back calculations from catch records by Reeves and Mitchell (1987b) conclude that this population numbered 800 to 1000 in the 1870's. Loss of mtDNA variation is expected to be rapid when population size is significantly reduced, because the smaller number of effective genes as compared to nuclear DNA makes it more sensitive to stochastic loss

through random drift (Birky *et al.* 1989). Among 11 samples, 5 different haplotypes were detected, including an A group haplotype. This level of variation may indicate that Ungava Bay is part of a larger population using the bay as a portion of their range, and that low numbers may suggest that rivers in Ungava Bay are not used as frequently as other locations. Alternatively, it is possible that the time since a bottleneck has not been long enough for mtDNA diversity to decrease appreciably.

Population Structuring of mtDNA

Comparisons of haplotype frequencies indicate that belugas in eastern Hudson Bay are genetically distinct with respect to belugas in Hudson Strait and Ungava Bay. Since all of these populations likely inhabit Hudson Strait in winter, and thus are not physically isolated, genetic differences must be maintained by philopatry to familiar estuaries. The return of females with young to the same estuaries each year would result in this population structuring of mtDNA markers. Such philopatry over seasons has indeed been observed for belugas (Finley *et al.* 1982; Caron and Smith 1990).

There has been, however, some gene flow between populations, as seen by the presence of A types in the northern populations. It is uncertain whether the B haplotypes seen in eastern Hudson Bay represent individuals from the northern populations, or from western Hudson Bay. Brennin (1992; Brennin *et al.*, in prep.) found mtDNA RFLP haplotypes within lineage II were found in many populations in western and higher arctic locations. These populations were concluded to be derived from populations which occupied the western waters of

the Arctic during the Wisconsin Ice Age. RFLP analysis did not differentiate between these stocks, so the origin of B types is unclear.

Calculation of nucleotide divergence, after correction for intrapopulation diversity (d_A), was 1.1% between eastern Hudson Bay and southern Hudson Strait/Ungava Bay populations. This value is within the range observed between species of primates and rodents (e.g. 0.18 - 4.1%; Wilson *et al.* 1985). Similar ranges of estimates have been found between populations of minke whales (3.9%, Wada and Numachi 1991) and humpback whales (2.8 - 3.1%; Baker *et al.* 1993).

Although several studies of mtDNA variation in cetacean populations (Schaeff *et al.* 1991; Baker *et al.* 1993; Rosel 1992) have used nucleotide divergence to calculate the time since populations have shared a common ancestor, this was not attempted for the present study. Measures of sequence divergence reflect the relatedness of the haplotypes themselves but not necessarily the relatedness of populations or species (Pamilo and Nei 1988). Recent gene flow between populations will also act to prevent accurate measures of divergence (Slatkin 1989).

Significant differences in haplotype frequencies between Nastapoka River and Little Whale/Great Whale indicate there may be different whales occupying these estuaries. Alternatively, the increased representation of B group haplotypes could have arisen through sampling error, as four of these whales were males. Chance sampling of pods containing males which are less philopatric, moving up and down the coast of Nunavik, could provide an unrepresentative bias within the small sample size for the Little Whale/Great Whale rivers (N = 17).

A more intriguing possibility is that the B-type whales sampled may in fact

be western Hudson Bay belugas which have remained in the eastern Hudson coast/ Belcher Island area. Based on patterns of seasonal ice coverage, P. Richard (personal communication) has proposed that belugas summering on both coasts move south down the eastern side of Hudson Bay. Ice breaks up along the eastern coast first, allowing eastern Hudson belugas to move into estuaries. Around May, access opens up to areas along James Bay and southern Hudson Bay, where substantial numbers of belugas are observed (Smith and Hammill 1986; Richard 1993). By mid-summer, whales can move into Churchill on the western coast of Hudson Bay. Until that time, belugas likely remain around the Belcher Islands. Haplotypes similar to both eastern and western Hudson Bay populations have been found among belugas taken at Sanikiluaq in the Belchers (J. Brown, personal communication). Whales with group B haplotypes may therefore represent individuals which have not yet returned to western coast estuaries, or may be descendants of a lineage of an ancestral female which relocated to eastern Hudson Bay. This could occur in the case of a pregnant female ceasing migration to calve, then using the estuary during that summer. Association with females philopatric to this region could result in recruitment of a "western" beluga into the population.

A final scenario involves the occupation of estuaries along the east, south and west coasts being occupied by different overlapping subsets of philopatric belugas. The trend of increasing "B-type" whale representation could continue along the coast. Brennin (1992; Brennin *et al.*, in prep.) has proposed that the western and eastern coasts of Hudson Bay were opened up separately to colonization by belugas from the north. This could result in a pattern by which most of each coast was colonized by different whales, but more southerly locations

became available gradually to both sides, resulting in increased levels of mixing towards the Ontario coast and James Bay. Belugas from these areas would have to be sampled before this can be tested.

Conclusions and Recommendations

Conservation of harvested populations should be directed toward management of individual stocks. This is particularly important given that estimates of sustainable yields (e.g Béland *et al.* 1988) made despite poor knowledge of vital rates (Doidge 1990) are viewed as having limited use in management (Doidge and Finley 1993). Genetic information can provide insight into the identity and character of these populations.

Sequence analysis of the mitochondrial control region in this study indicates strongly that whales hunted in eastern Hudson Bay in the summer belong to a stock which is genetically distinct from those summering in Southern Hudson Strait and Ungava Bay. The data also indicates there is probably some recruitment into both populations, certainly from eastern Hudson Bay to southern Hudson Strait. Whether this is bidirectional exchange is unclear, given that there is a source of mtDNA genotypes in western Hudson Bay which are similar to those in Hudson Strait. The evolutionary relationships among these northern and western populations has yet to be determined, aside from their probable past history as a single "western" Arctic stock (Brennin 1992; Brennin *et al.*, in prep.). Nonetheless, management strategies should be considered independently for these two regions. Quotas are currently assessed per community for all of Nunavik (D.W. Doidge, pers. comm.). This study suggests that villages in the northern

and eastern areas of Nunavik harvest from different resources.

The results presented in this study also suggest there may be some differences between whales taken at the Nastapoka River and those from more southerly locations in Hudson Bay. Further, there may be a moderate level of recruitment to these areas from the western Hudson Bay population by way of the Belcher Islands. Increased sample sizes as well as direct comparisons to belugas taken at Belcher Island are required to evaluate this possibility.

Observations of haplotype composition of single-day multiple captures within Nastapoka suggest that pods of whales found inhabiting estuaries may consist of highly related individuals within a limited number (e.g. one or two) of matrilines, although there are some less related animals present, including some from "group B"-dominated populations. This could be tested using nuclear markers such as microsatellite loci, which have been used in natural populations to trace relatedness and identify parentage (Gotelli *et al.* 1993; Amos *et al.* 1993; Bruford and Wayne 1993).

This study has shown that there is likely some extent of specific estuarine use, and partial to highly nonoverlapping populations of whales occupying estuaries along the northern and eastern Nunavik coasts, particularly between Hudson Bay and Hudson Strait. No substantial differences were found between Hudson Strait and Ungava Bay belugas, but it is uncertain whether this reflects usage of the Ungava estuaries by other populations, or an artifact of a recent population bottleneck. The lack of replacement of belugas in areas such as the Great Whale River, which once boasted a high concentration of belugas (Reeves and Mitchell 1989), is consistent with strong philopatry to estuaries suggested by

this and previous work (Brennin 1992; Brennin *et al.*, in prep.). If the diversity of the whole population is subdivided into genetically differentiated demes, then local extinctions may result in the loss of variation specific to that region. Therefore, conservation should target the distinct populations individually as exclusive entities not likely to be replaced if lost. However, further work is required to clarify the inter-estuarine differences within the major differentiated populations identified in this study.

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INTRODUCTION

Investigations into the genetic structure of populations have predominantly featured the use of mitochondrial DNA (mtDNA) marker analysis. With its uniparental, clonal mode of inheritance (Dawid and Blacker 1972; Hutchison *et al.* 1974) and high rate of mutation (Brown *et al.* 1979; Wilson *et al.* 1985; Cann *et al.* 1987), it serves as a powerful tool for evolutionary study.

Despite its utility in phylogeographic studies of natural populations, mitochondrial DNA does not provide a complete assessment of their genetic relatedness. Lack of recombination results in a mitochondrial genome consisting of a set of completely linked loci, and thus effectively only one locus (Wilson *et al.* 1985). Phylogenetic assessments made from a single locus may not accurately represent the genetic history of a population or species (Avise *et al.* 1984, 1987; Wilson *et al.* 1985). Further, due to its uniparental mode of transmission (Dawid and Blacker 1972, Hutchison *et al.* 1974), mitochondrial DNA will show the genetic population structure of maternal lineages only (Pamilo and Nei 1988; Cronin 1993). Populations which are determined to be genetically distinct on the basis of mtDNA may in fact be connected by gene flow through males. Differences in dispersal may mean population structure of maternally inherited mtDNA may differ from that of biparentally inherited ncDNA (Karl and Avise 1992; Karl *et al.* 1992). Several studies have shown a better understanding of

relationships of populations or closely related species is gained by combined analyses of both genomes than with either type alone (Avise and Saunders 1984; Dowling and Brown 1989; Baker *et al.* 1994).

Minisatellites, also called variable number of tandem repeat (VNTR) loci (Nakamura *et al.* 1987), are segments of DNA which consist of multiple copies of a short sequence (typically 10-60 bp). They are widely spread throughout the genomes of organisms, although they are preferentially located at the ends of chromosomes (Royle *et al.* 1988). These loci are hypervariable, with many alleles occurring at a single locus, as a result of a high mutation rate for the loss and gain of repeat units (Jeffreys *et al.* 1985a; Wong *et al.* 1986; Gyllenstein *et al.* 1989). Mutation appears to occur either through replication slippage, unequal sister chromatid exchange or gene conversion (Wolff *et al.* 1989; Jeffreys *et al.* 1994).

DNA fingerprinting involves the use of conserved "core" sequences, to simultaneously detect a large number of independently segregating hypervariable loci. The first of these polycore probes, 33.15 and 33.6, were characterized by Jeffreys *et al.* (1985a,b), who found that the resultant patterns were so variable as to be individual-specific. 33.15 and 33.6 vary in length and precise sequence of the core repeat (Jeffreys *et al.* 1985a,b), and have been shown to detect independent sets of variable fragments (e.g. 1% co-detection, Jeffreys *et al.* 1986, 1991). Linkage and allelism of fragments are thought to be low because of the widespread nature and enormous allelic length variability. Jeffreys *et al.* (1991) found less than 10% of bands to be linked or allelic in human pedigrees, due to most alleles at most loci being small and therefore not within the normal range of scoring (> 2 kb). This is not necessarily true for all species, however, as clear

evidence of major linkage between fragments has been detected in mice (Jeffreys *et al.* 1987), as well as multifragment alleles in parrots (Brock and White 1991).

Multilocus fingerprinting relies on two key features of minisatellite loci. Firstly, fingerprints show Mendelian inheritance (Jeffreys *et al.* 1986). On average, individuals will receive half of their bands from each parent (Jeffreys *et al.* 1985a). Secondly, the probability that two unrelated individuals will present the same banding pattern is generally many orders of magnitude smaller than the reciprocal of the population size (Jeffreys *et al.* 1985a; Burke *et al.* 1991). The use of fingerprinting involves assumptions regarding constant allele frequencies and statistical independence of all bands. In fact, bandsharing will decrease with increased fragment size (Jeffreys *et al.* 1985b), and non-independence can occur through linkage or allelism of bands as noted above.

Most studies utilizing multilocus fingerprinting have been directed at measuring of familial relationship in natural populations such as paternity (e.g. in lions, Gilbert *et al.* 1991; birds, Burke and Bruford 1987; Gibbs *et al.* 1990; Jamieson *et al.* 1995; pilot whales, Amos *et al.* 1991a,b; and seals, Harris *et al.* 1991) and relatedness (e.g. Brock and White 1987; Hoelzel and Dover 1989; Lehman *et al.* 1992). More recently, however, fingerprinting has been increasingly used for population-level analyses. Several empirical studies have produced reliable estimates of genetic diversity (Gilbert *et al.* 1990, 1991; Jeffreys and Morton 1987; Menotti-Raymond and O'Brien 1990) and detected low levels of variation attributed to inbreeding (Faulkes *et al.* 1990; Reeve *et al.* 1990; Gilbert *et al.* 1990, 1991; Triggs *et al.* 1992; Timms *et al.* 1993; Patenaude *et al.* 1994).

These results have contributed to a view that minisatellites may have the potential to describe population subdivisions that may exist on timescales too brief to be measured by conventional allozyme or single locus RFLP markers (Burke and Bruford 1987; Gilbert *et al.* 1990; Packer *et al.* 1991). Features of minisatellites such as a wide range of heterozygosities (Jarman and Wells 1989), high mutation rates, and the dependence of allele frequencies on the rate of generation and rate of loss by drift make them potentially appropriate to examine genetic variation within and between closely related populations (Jarman and Wells 1985; Degnan 1993) and to compare patterns of nuclear gene flow and those of mitochondrial gene flow as shown by mtDNA. Several authors have reported the detection of significant differences of within- and between-population variation using multilocus fingerprinting techniques (Hoelzel and Dover 1991; Degnan 1993; Robinson *et al.* 1993; Rave 1995).

Beluga whale populations inhabiting arctic and subarctic waters in Canada are recognized as subdivided into six populations based upon geographic discontinuity or morphometric differences: Cumberland Sound/Southeast Baffin, eastern Hudson Bay, western Hudson Bay, high Arctic, Beaufort Sea and the St. Lawrence (Sergeant and Brodie 1975). The splitting of a once presumably continuous range is likely the result of the separation of populations for about 50,000 years during the Wisconsin ice age by the Laurentide ice sheet (Denton and Hughes 1981) and the subsequent adaptation to local habitat. However, these populations have remained segregated, despite the lack of a physical barrier, since recession of the ice sheet approximately 7,000 years ago.

Maintenance of beluga distributions is believed to be due to philopatric

behaviour. Belugas have been observed to return to the same estuaries throughout the summer and in successive summers (Finley *et al.* 1982; Caron and Smith 1990). Philopatry of females and calves has been observed to be the basis for population structuring of several cetacean species (e.g. in humpbacks, Clapham and Mayo 1990, Baker *et al.* 1990, 1993; right whales, Schaeff *et al.* 1993). Studies showing differences in mtDNA RFLP haplotype distributions between eastern and western Hudson Bay beluga populations indicates this is the case for beluga populations (Brennin 1992; Brennin *et al.*, in prep.).

However, as previously discussed, mtDNA does not provide the complete picture of the genetic relationship of these stocks. Gene flow connecting populations may occur if genetic exchange occurs via migration or interbreeding. Belugas in on both coasts of Hudson Bay are believed to intermingle on a common wintering ground in Hudson Strait for a period which spans the probable mating season (Brodie 1971; Sergeant 1973), thus providing ample opportunity for exchange (Finley *et al.* 1982; Richard *et al.* 1990).

The aim of this study was to assess the extent of gene flow between eastern and western Hudson Bay populations through the analysis of ncDNA minisatellite marker loci. This was addressed by comparing levels of bandsharing for intra-and interpopulation comparisons. A high degree of gene flow through males would be reflected in a lack of significant differences of within- and between-population comparisons.

DNA fingerprinting has previously been applied in genetic analyses of beluga populations in the St. Lawrence estuary and the Mackenzie Delta (Patenaude *et al.* 1994). Bandsharing in the St. Lawrence population was found

to be significantly higher than in the Mackenzie Delta/Beaufort Sea population, indicating a reduced level of genetic diversity within the St. Lawrence population. This finding is consistent with the hypothesis that this small, isolated population has not recovered from earlier depletion (see Reeves and Mitchell 1984; Pippard 1985) due in part to reduced genetic variation (Patenaude *et al.* 1994). St. Lawrence and Mackenzie Delta belugas were also used in this study, for purposes of comparison to the findings of Patenaude *et al.* (1994), and as populations that have been separated in past and present by physical barriers and geographic distance. Data from mitochondrial DNA analyses have not indicated genetic differences between St. Lawrence and eastern Hudson Bay, nor between Mackenzie Delta and western Hudson Bay (Brennin 1992; Brennin *et al.*, in prep.). Thus comparisons were made between these pairs to determine whether DNA fingerprinting could detect any differences.

MATERIALS AND METHODS

Sample Collection

Beluga samples were collected over several years at various locations in the eastern and western Hudson Bay, and the Mackenzie Delta, from Inuit and Inuvialuit kills, through the co-operation of the Department of Fisheries and Oceans, Makivik Corporation, and the Fisheries Joint Management Committee. Samples from the St. Lawrence Estuary were obtained from dead animals washed ashore and collected for autopsies by the Institut National d'Ecotoxicologie du Saint-Laurent. Tissues (skin, heart, muscle, liver) were shipped frozen or preserved in a NaCl-saturated solution containing 20% DMSO and 0.25 M EDTA (Seutin *et al.* 1991), and stored at -20°C upon arrival.

DNA Extraction and Quantification

Tissue samples (0.50-0.75g) were ground to a fine powder in liquid nitrogen and suspended in 3.5 mL lysis buffer (5 M urea, 0.2 M NaCl, 0.1 M Tris-HCl, pH 8.0, 0.5% *n*-laurylsarcosine, 10 mM EDTA), and incubated for one to three weeks at 37°C. During this time samples were subjected to digestion by proteinase K (83 units), either twice at 37°C for 12 h, or once at 55°C for 90 min followed by 12 h at 37°C. DNA was extracted twice with phenol and chloroform (70:30) and once with chloroform, and then precipitated by adding sodium acetate

to a final concentration of 0.15 M and two volumes of cold 95% ethanol. DNA pellets were washed with 70% ethanol, air-dried and dissolved overnight in 0.5-1.0 mL TNE_2 (10 mM Tris-HCl, 10 mM $NaCl_2$, 2 mM EDTA, pH 8.0) at 37°C.

The amount of DNA was determined using the TKO 100 DNA Mini-Fluorometer (Hoefner), which assays DNA concentration based on specific binding of Hoechst 33258 fluorescent dye to A-T rich regions of DNA (Cesarone *et al.* 1979). This estimate was then adjusted by comparison with a standard of known concentration on agarose gels. Gels were stained with ethidium bromide and visualized under shortwave UV light.

DNA Fingerprints

Genomic DNA (5 μg) was digested with restriction enzyme *Hae*III (3 units/μg of DNA) according to the manufacturer's instructions (Bethesda Research Laboratories). Lambda DNA restriction fragments were added to each sample as a size marker and as an internal control for differential mobility (Brock & White 1991; Galbraith *et al.* 1991). DNA fragments were electophoretically separated on 28 cm long, 0.8% agarose gels in TBE buffer (45 mM Tris-borate (pH 8.0), 1 mM EDTA). Gels were run at constant voltage (75-80V) for $\approx$ 40 h, with buffer replacement after 24 h. Gels were stained with ethidium bromide and visualized under shortwave UV light, trimmed to desired size, and depurinated in 0.25 N HCl for 15 min. This was followed by denaturing in 0.5 M NaOH, 1.5 M NaCl for 50 min, and neutralization in 1.5 M NaCl, 0.5 M Tris-HCl, 0.001 M EDTA, pH 7.2 for 50 min. DNA was transferred to a positively charged polyvinylidene difluoride membrane (Immobilon-N, Millipore Corporation) by

capillary flow (Southern 1975). Five fingerprint blots were prepared, each containing DNA from four individuals of each population, loaded in random order.

The blots were prehybridized overnight at 65°C in a solution of 7% SDS, 1 mM EDTA (pH 8.0), 1% bovine serum albumin, 0.25 M sodium ortho-phosphate (pH 7.2) (Westneat *et al.* 1988). Probe DNA (25 ng) was radioactively labelled with 50 μCi [α-^{32}P] dCTP by random primer extension (Feinberg and Vogelstein 1983) to a specific activity of at least 5 x 10^8 dpm/μg. Blots were hybridized overnight at 65°C with Jeffreys's 33.6 or 3.15 minisatellite probes (Jeffreys *et al.* 1985a), along with similarly-labelled adenovirus marker DNA. Blots were washed three times (5 min, 30 min, and 20 min) at 65°C in 2 x SSC, 0.1% SDS. Blots were exposed to Dupont Cronex X-ray film and a Cronex Lightning Plus intensifying screen for 1-10 days, then to Kodak XAR5 film for 14 days. Blots were stripped in 0.4 NaOH at 42°C for 45 min prior to reprobing. Finally, hybridization with [α-^{32}P] dCTP-labelled λ was carried out under the same conditions as described above.

DNA Fingerprint Analysis

Fingerprints were scored for the region between the largest λ marker band (21 kb) and the 3.5 kb marker band. DNA fragments were identified by measuring the distance migrated relative to the closest internal λ size marker (Galbraith *et al.* 1991). A band was considered identical in two individuals if the migration distances were within 0.5 mm. Fragments that were less than half the intensity of their comigrating counterparts (after correction for variation in DNA amount between lanes) were considered to be different fragments (Bruford *et al.*

1992). Such bands were not scored as they could not be detected in samples having the more intense band.

DNA fingerprint bandsharing coefficients, D, were calculated as

$$D = 2N_{ab}/ (N_a + N_b)$$

where N_{ab} is the number of bands shared between two individuals and N_a and N_b are the total number of bands scored in each individual (Wetton *et al.* 1987). Fingerprints were run at a standard distance to facilitate inter-blot comparisons; however, scoring of bandsharing coefficients was limited to samples run on the same blot. Scoring was performed without knowledge of the samples. Mean bandsharing coefficients within and between populations were calculated for each probe by combining data from all five blots.

Bandsharing coefficients calculated for pairwise combinations of animals result in nonindependent observations. To account for interdependence, Mantel tests (NTSYS; Rohlf 1990) were used to determine differences in mean bandsharing coefficients for within- and between-population comparisons. Two symmetrical similarity matrices consisting of bandsharing coefficients for each probe and their corresponding comparison designation (within- or between-population) or population of origin were compared using the Mantel test statistic Z (NTSYS; Rohlf 1990). Approximate t-values were then calculated and compared with the standard t-distribution with infinite degrees of freedom (Schnell *et al.* 1985) and by comparing Z-values with 9,999 permutational distributions (Rohlf 1990).

RESULTS

DNA Fingerprints

*Hae*III-digested DNA from belugas from four summering (or resident, in the case of the St. Lawrence) populations were analyzed by DNA fingerprinting using multilocus minisatellite probes Jeffreys 33.15 and 33.6 (Jeffreys *et al.* 1985a). Both probes gave fingerprints of good clarity and intensity (Fig 2.1). *Hae*III was chosen because it gave the best scorable fingerprints in a prior study (Patenaude *et al.* 1994).

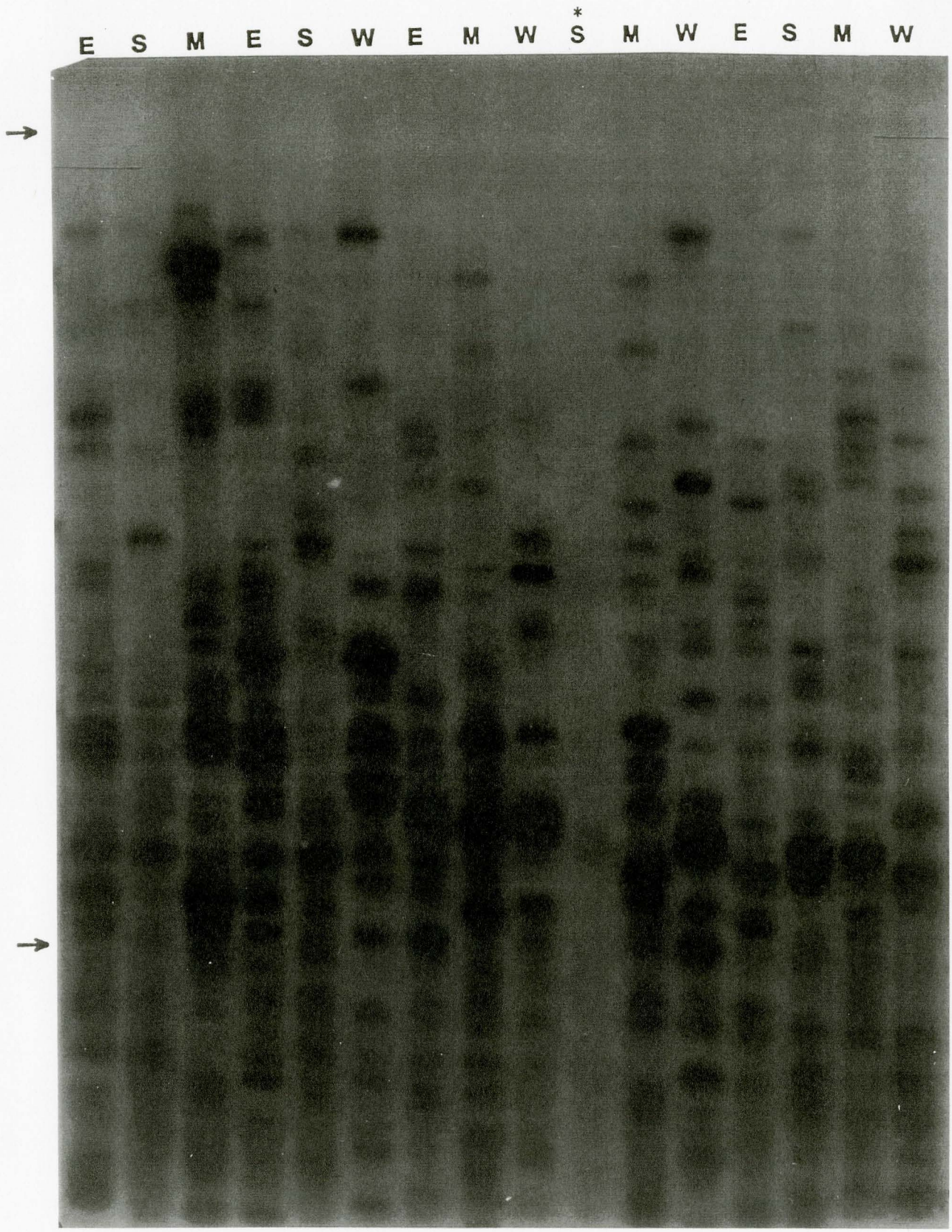
The mean numbers of scored bands for each probe were comparable to those found in other studies (Jeffreys *et al.* 1987; Burke and Bruford 1987; Wetton *et al.* 1987) but lower than in the previous beluga study (Patenaude *et al.* 1994), probably due to differing scored regions and band separation. The mean number of bands was 14.6 (S.D. = 2.5) for 33.15 and 17.5 (S.D. = 2.9) for 33.6. Four individuals from the St. Lawrence Estuary gave profiles of limited use due to the highly degraded nature of the DNA. These were removed from analysis.

Average bandsharing coefficients for each fingerprint probed with Jeffreys 33.15 ranged considerably, from 0.092 to 0.156. Autoradiographs were examined for sharpness of bands and intensity. No obvious differences in clarity were observed; however, there were some interblot differences in banding intensity over some portions of the scored area, as well as in the total number of bands scored.

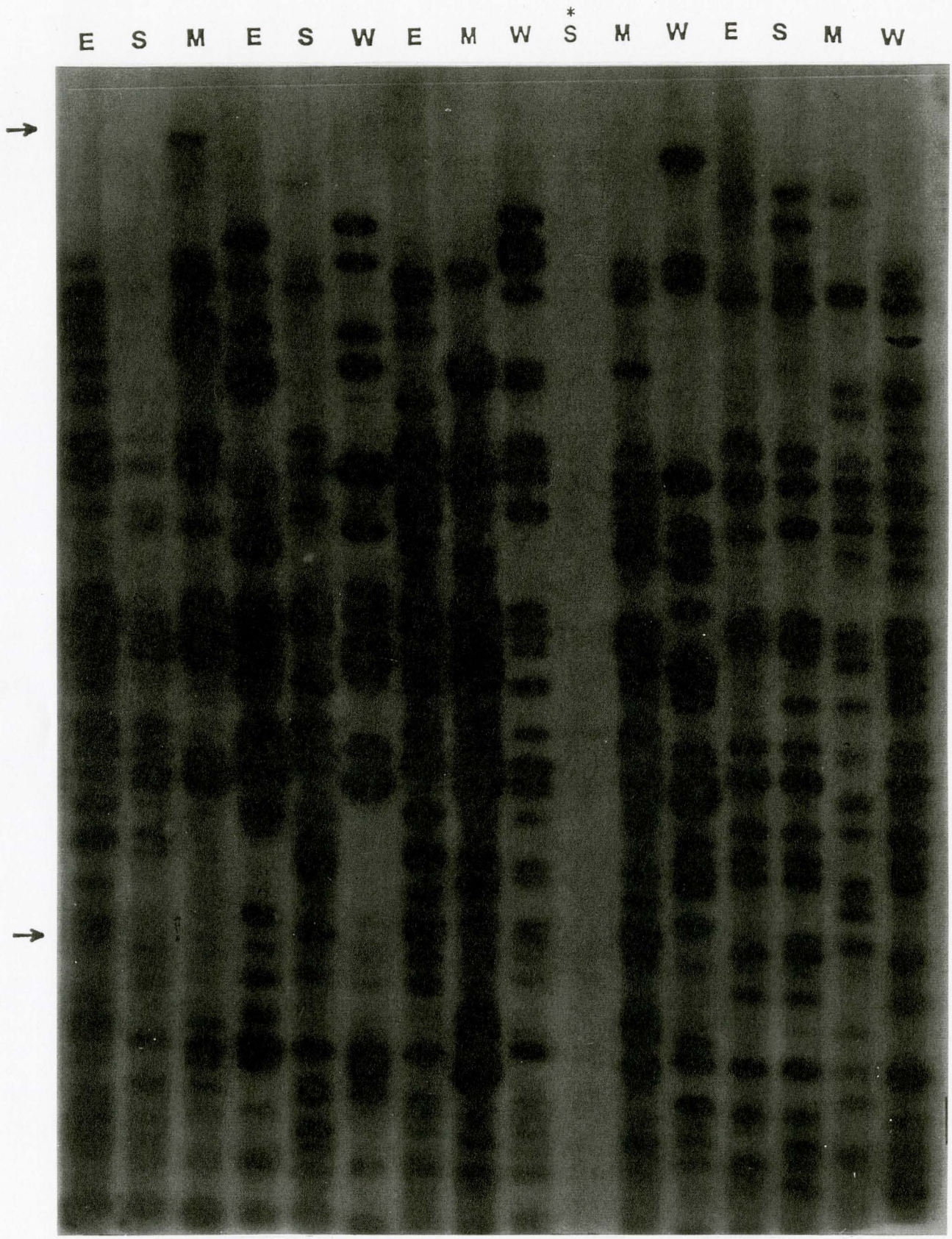
FIGURE 2.1. Multilocus DNA fingerprints of genomic DNA from St. Lawrence (S), Mackenzie Delta (M), eastern Hudson Bay (E) and Western Hudson Bay (W) belugas digested with *Hae*III and probed with (a) Jeffreys 33.15 and (b) Jeffreys 33.6.

* indicates lane not scored because of poor quality of fingerprint.
→ scoring range for bandsharing coefficients

(a)



(b)



Mean overall bandsharing over for Jeffreys 33.6 varied less, ranging from 0.183 to 0.214.

Intrapopulation Bandsharing

Bandsharing coefficients generated for within-population comparisons fell in the lower range of those found in other population studies (Jeffreys *et al.* 1985b; Burke and Bruford 1987; Wetton *et al.* 1987). However, the absolute mean bandsharing values for the St. Lawrence and Mackenzie Delta were lower than found by Patenaude *et al.* (1994), although the relative differences between the two were similar. Mean bandsharing values with Jeffreys 33.6 were higher than with 33.15 (Table 2.1).

Bandsharing was highest for both probes within the St. Lawrence population (0.229 with 33.15; 0.370 with 33.6), indicating a decrease in genetic diversity relative to other beluga populations. *D*-scores were lowest for 33.15 in Western Hudson Bay (0.089) and for 33.6 in Eastern Hudson Bay (0.192). The low value for western Hudson Bay is much lower than the typical range of diverse populations (0.10 - 0.35; e.g. Jeffreys *et al.* 1985b; Burke and Bruford 1987). Mean *D*-scores, and thus diversity, for other Mackenzie Delta and Hudson Bay combined were intermediate in both cases.

The value generated for Western Hudson Bay animals probed with Jeffreys 33.15 was surprisingly low, and closer analysis revealed that mean bandsharing for this population was lower than average for all blots. Fingerprints were examined to assess the quality of these samples. Lanes containing DNA from western Hudson Bay samples did not differ from the remainder in terms of visual

TABLE 2.1. Summary statistics for fingerprint analysis of populations of beluga whales in Canadian waters using Jeffreys 33.15 and 33.6.

	33.15		33.6	
Within	N ± s.d.	BSC ± SE	N ± s.d.	BSC ± SE
SL[1]	13.9 ± 2.6	0.229 ± 0.024	15.3 ± 1.7	0.370 ± 0.036
EH	15.4 ± 2.9	0.160 ± 0.017	17.9 ± 3.0	0.192 ± 0.022
WB	14.3 ± 2.4	0.089 ± 0.014	17.9 ± 2.9	0.222 ± 0.020
MD	14.7 ± 2.1	0.137 ± 0.017	18.3 ± 2.9	0.210 ± 0.018
HB	14.8 ± 2.7	0.134 ± 0.008	17.6 ± 3.1	0.212 ± 0.008
Between				
SL-EH	—	0.141 ± 0.012	—	0.215 ± 0.019
SL-WB	—	0.128 ± 0.011	—	0.203 ± 0.019
SL-MD	—	0.133 ± 0.014	—	0.123 ± 0.099
EH-WB	—	0.144 ± 0.011	—	0.215 ± 0.015
EH-MD	—	0.132 ± 0.014	—	0.176 ± 0.011
WB-MD	—	0.171 ± 0.021	—	0.192 ± 0.009
SL-HB	—	0.134 ± 0.008	—	0.209 ± 0.013
MD-HB	—	0.122 ± 0.008	—	0.185 ± 0.007

[1]Abbreviations: SL - St. Lawrence Estuary; HB - Hudson Bay; MD - Mackenzie Delta; EH - Eastern Hudson Bay; WB - Western Hudson Bay.
N = mean bands scored, BSC = bandsharing coefficient.

clarity or band sharpness. Mean bands scored per individual did not differ considerably from averages for all blots.

The frequency distributions of pairwise bandsharing coefficients for within-population comparisons showed the higher levels for the St. Lawrence population compared to all other populations (Fig. 2.2). Distributions also showed Western Hudson Bay values to be lower than those for other populations. Otherwise, distribution patterns are conflicting or do not appear different. For example, bandsharing for 33.15 appears higher for eastern Hudson Bay compared to western Hudson Bay, while the converse is true for 33.6.

Mantel tests of matrix comparison found bandsharing in the St. Lawrence population to be significantly higher than all other populations (Table 2.2), consistent with a previous study. (Patenaude *et al.* 1994). However, comparisons of within-population similarity between other populations were conflicting. Significant differences were found for all comparisons, although the data from the two probes were not concordant with respect to higher or lower levels of similarity.

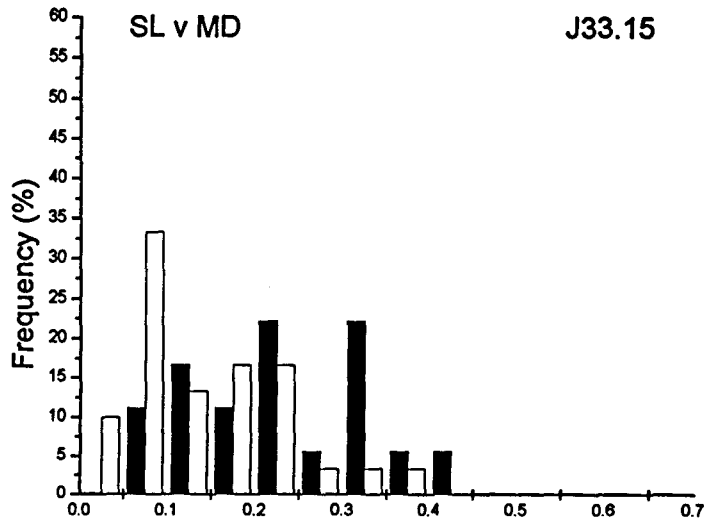
Interpopulation Comparisons

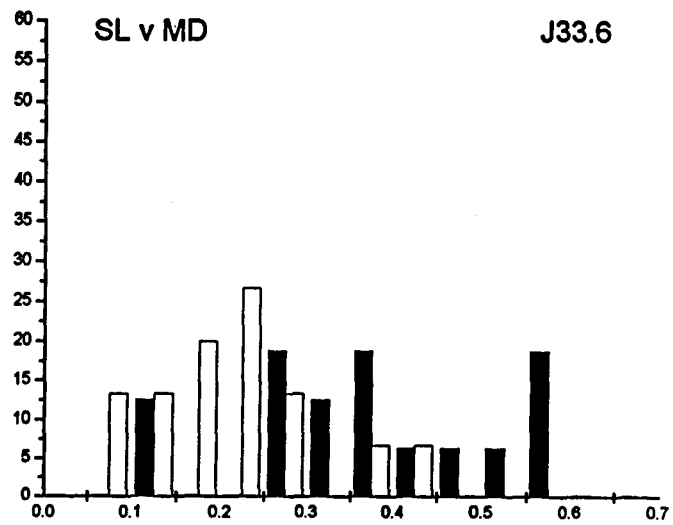
Bandsharing coefficients for between-population comparisons were similar in range to those for within-population estimates. Estimates were higher for Jeffreys 33.6 compared to 33.15.

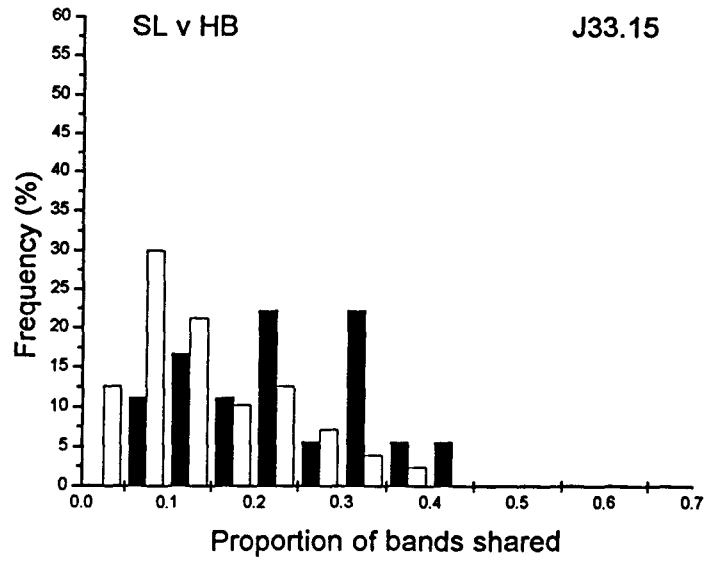
Bandsharing patterns were compared for within- and between-population data sets in an attempt to detect genetic isolation of beluga populations. Frequency distributions of *D*-scores show higher similarity within the St. Lawrence

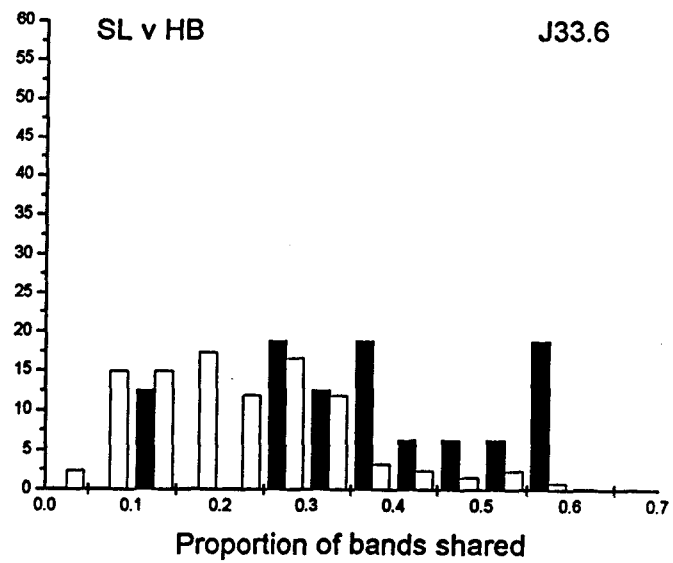
FIGURE 2.2. Frequency distributions of pairwise bandsharing coefficients for within-population comparisons. Black and white bars represent the first and second populations listed in the header, respectively.

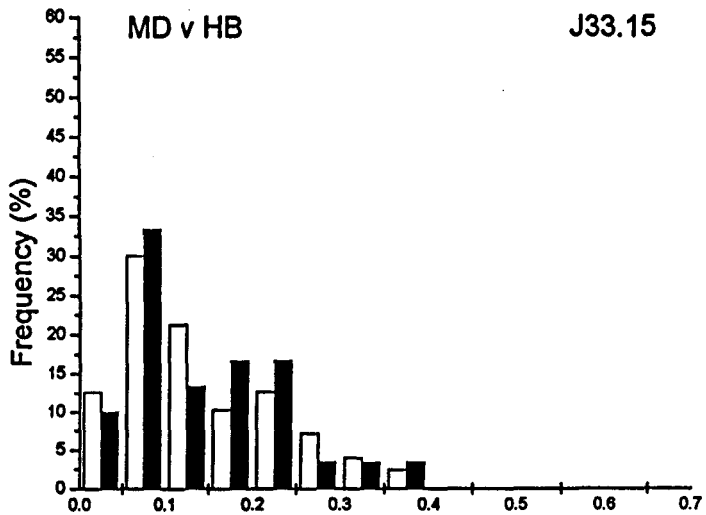
Abbreviations: SL = St. Lawrence Estuary; HB = Hudson Bay;
EH = Eastern Hudson Bay; WH = Western Hudson Bay; MD = Mackenzie Delta.

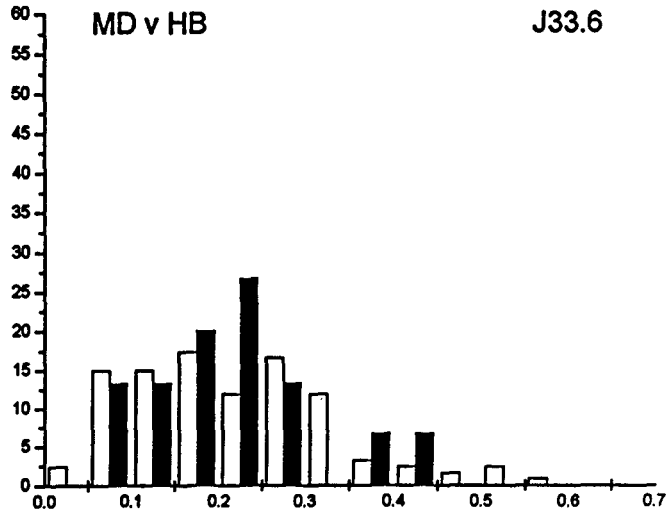

SL v MD
J33.15
Frequency (%)

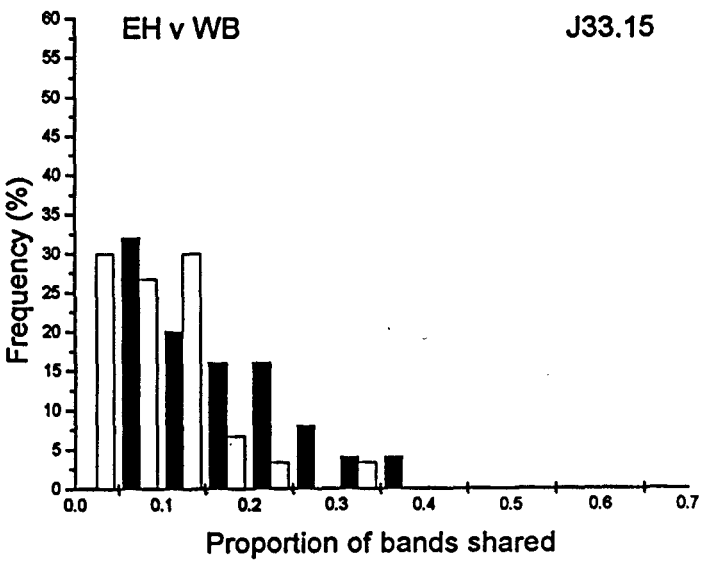

SL v MD
J33.6


SL v HB
J33.15
Frequency (%)
Proportion of bands shared


SL v HB
J33.6
Proportion of bands shared


MD v HB
J33.15
Frequency (%)
0 5 10 15 20 25 30 35 40 45 50 55 60
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7


MD v HB
J33.6
0 5 10 15 20 25 30 35 40 45 50 55 60
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7


EH v WB
J33.15
Frequency (%)
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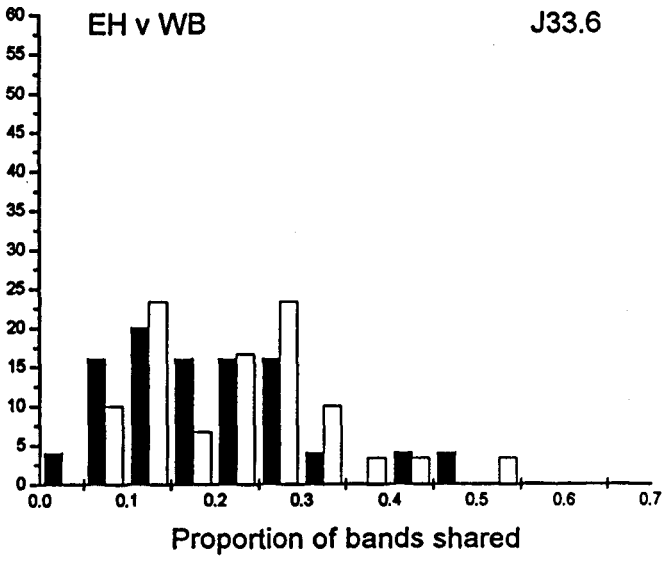
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Proportion of bands shared


EH v WB
J33.6
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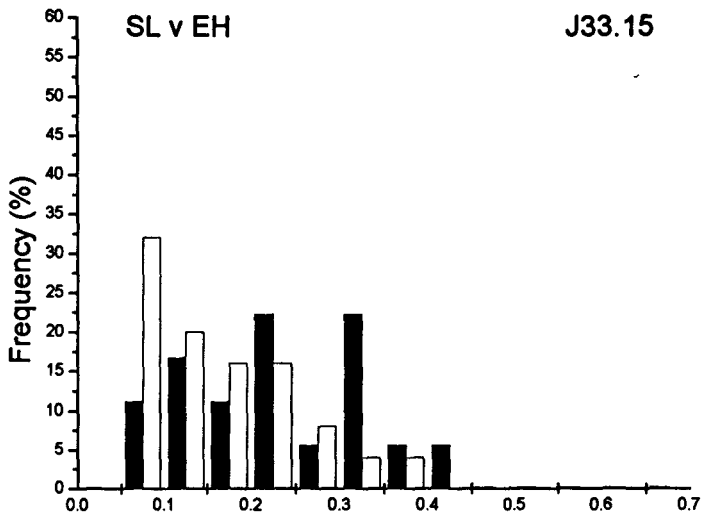
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Proportion of bands shared


SL v EH
J33.15
Frequency (%)
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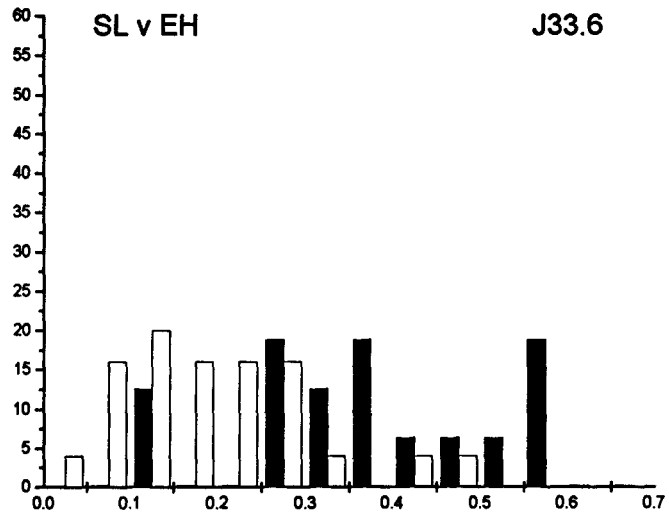
SL v EH
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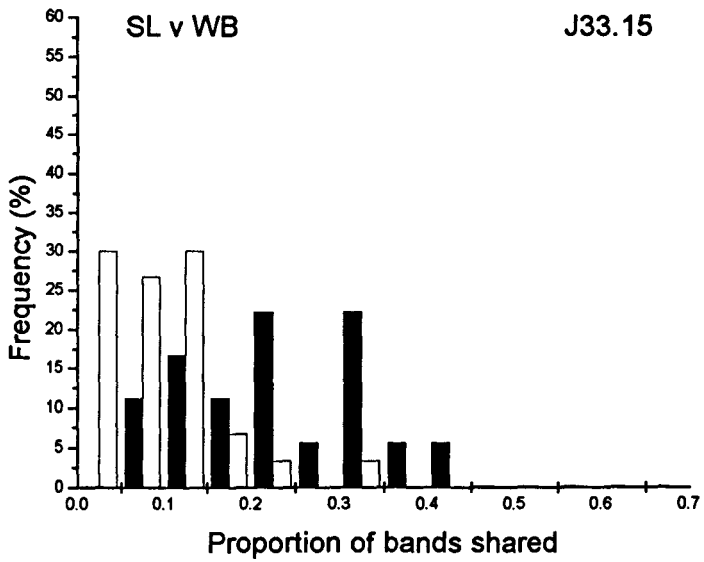
SL v WB
J33.15
Frequency (%)
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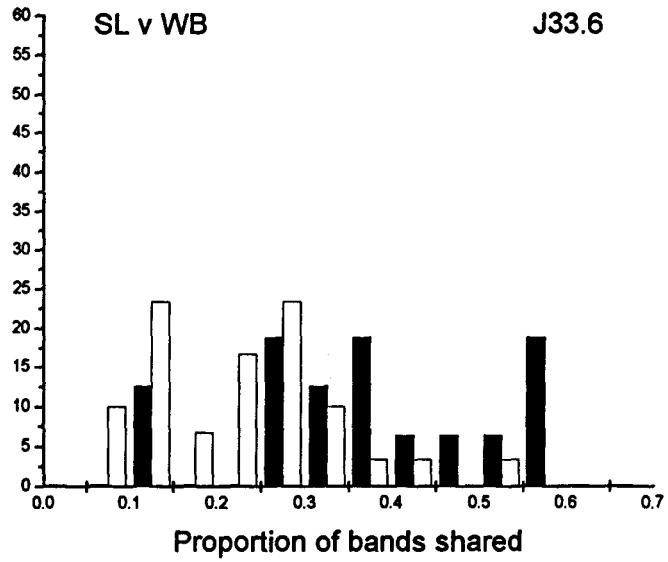
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Proportion of bands shared


SL v WB
J33.6
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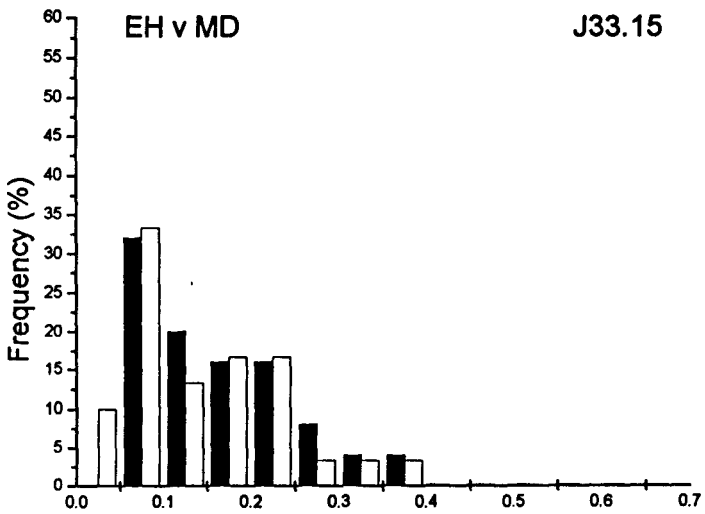
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Proportion of bands shared


EH v MD
J33.15
Frequency (%)
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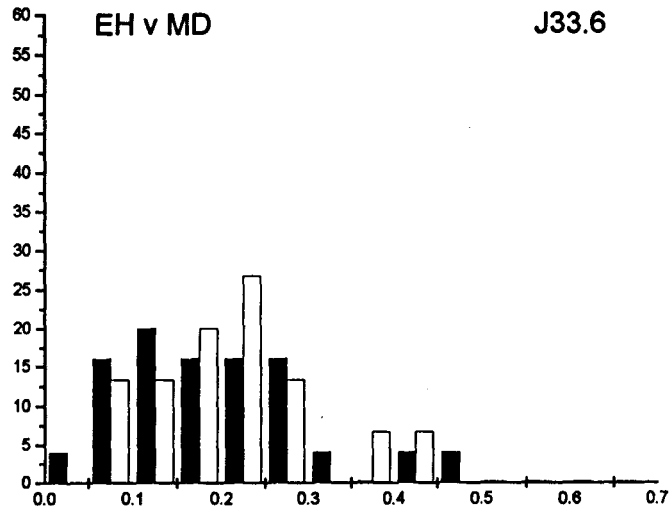
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7


EH v MD
J33.6
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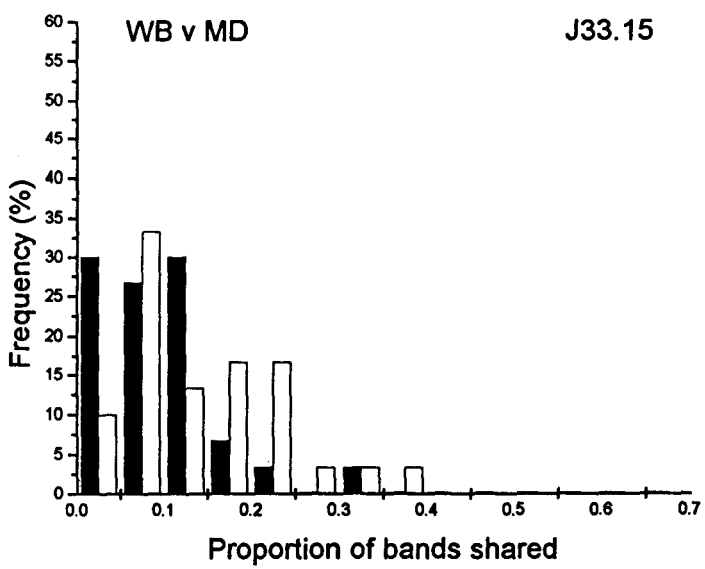
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7


WB v MD
J33.15
Frequency (%)
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0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Proportion of bands shared

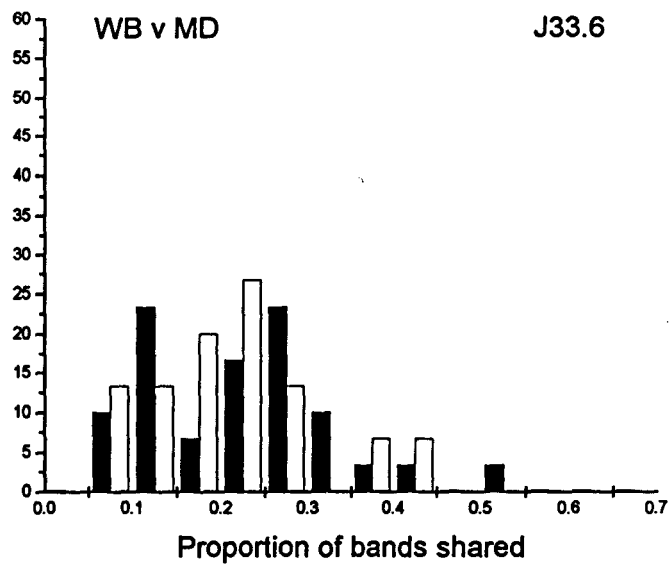

WB v MD
J33.6
0 5 10 15 20 25 30 35 40 45 50 55 60
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Proportion of bands shared

TABLE 2.2. Mantel tests for significant differences between within-population bandsharing coefficients for beluga whales in Canadian waters. Numbers represent probabilities associated with 9999 matrix permutations.

	J33.15		J33.6	
Major Populations				
SL v HB[1]	SL > HB	0.0001	SL > HB	0.0001
SL V MD	SL > MD	0.0001	SL > MD	0.0001
HB v MD	MD > HB	0.3122	HB > MD	0.3829
EH v WB	EH > WB	0.0001	WB > EH	0.0152
St. Lawrence vs. Hudson Bay				
SL V EH	SL > EH	0.0001	SL > EH	0.0001
SL v WB	SL > WB	0.0001	SL > WB	0.0001
Hudson Bay vs. Mackenzie Delta				
EH v MD	EH > MD	0.0001	MD > EH	0.0134
WB v MD	MD > WB	0.0001	WB > MD	0.0491

[1]Abbreviations: SL - St. Lawrence Estuary; HB - Hudson Bay; MD - Mackenzie Delta; EH - Eastern Hudson Bay; WH - Western Hudson Bay.

population than for comparisons of St. Lawrence belugas to belugas in Mackenzie Delta and Hudson Bay (Fig. 2.3), indicating that the St. Lawrence population is reproductively isolated from these populations.

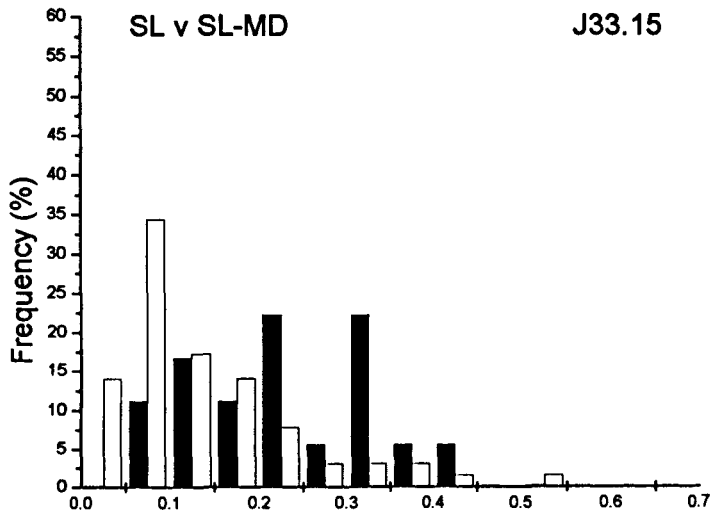
Mantel tests were used to assess the significance of differences in bandsharing between intra- and interpopulational comparisons (Table 2.3). St. Lawrence *D*-scores were significantly higher than in comparisons of St. Lawrence to Mackenzie Delta and to Hudson Bay belugas for both probes, suggesting some alleles are progressing toward fixation as a result of isolation of this small population from the Arctic stocks. Similarly, within-population bandsharing was higher for both Mackenzie Delta and Hudson Bay than for comparisons between the two populations. These observations are consistent with reproductively isolated populations.

Mantel tests of within-Hudson Bay *D*-scores to those between Hudson Bay and St. Lawrence, however, did not show significantly higher within-population values. This is probably due (in the case of Jeffreys 33.15) to the influence of low Western Hudson Bay bandsharing driving down the Hudson Bay combined average.

To test this, Mantel comparisons were performed using separate eastern and western Hudson Bay data sets. All comparisons showed intrapopulational western Hudson Bay mean pairwise bandsharing values to be significantly lower than those between western Hudson Bay and other populations for Jeffreys 33.15. Similarly, eastern Hudson Bay showed lower within-population Jeffreys 33.6 *D*-scores than those for comparisons between eastern Hudson Bay and the St. Lawrence. These results indicate that the lack of significance for Hudson Bay to Hudson Bay-St.

FIGURE 2.3. Frequency distributions of pairwise bandsharing coefficients for within- (black bars) and between-population (white bars) comparisons.

Abbreviations: SL = St.Lawrence Estuary; HB = Hudson Bay;
MD = Mackenzie Delta.


SL v SL-MD
J33.15
Frequency (%)
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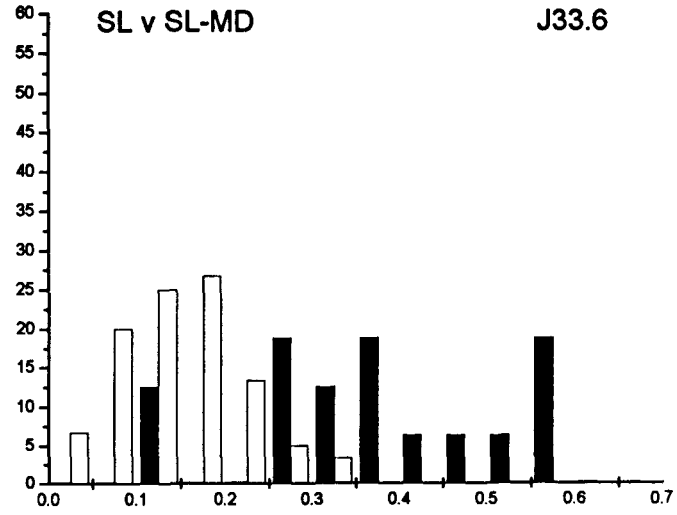
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7


SL v SL-MD
J33.6
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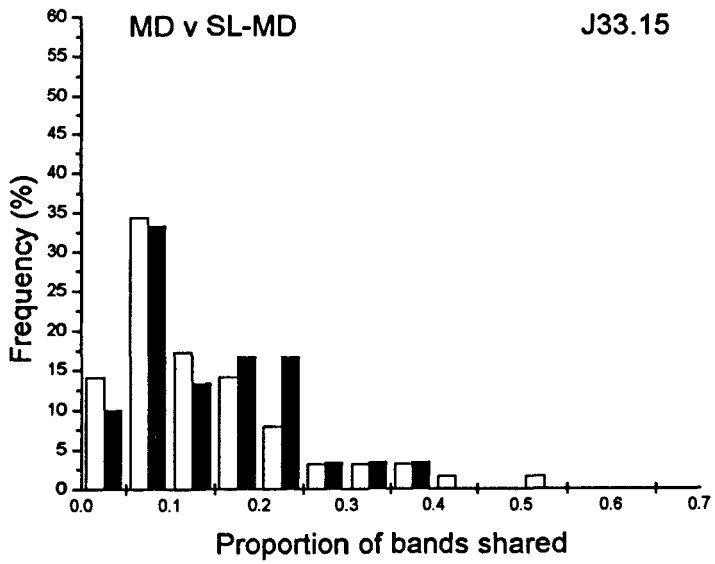
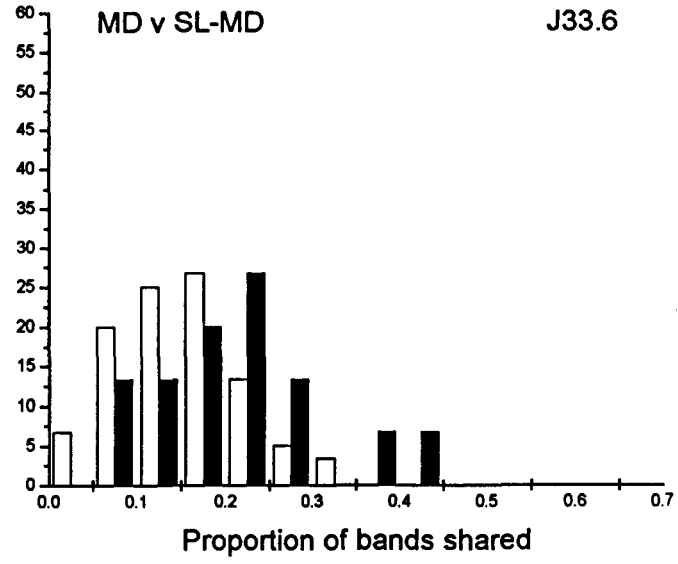
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7


MD v SL-MD
J33.15
Frequency (%)
0 5 10 15 20 25 30 35 40 45 50 55 60
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Proportion of bands shared


MD v SL-MD
J33.6
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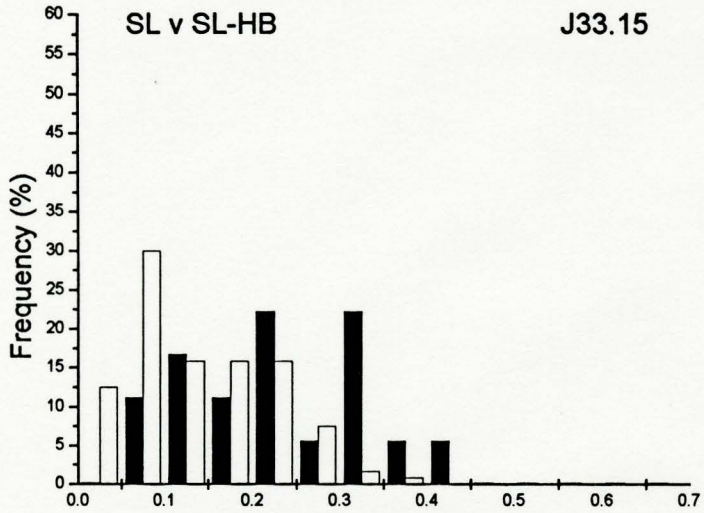
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Proportion of bands shared


SL v SL-HB
J33.15
Frequency (%)
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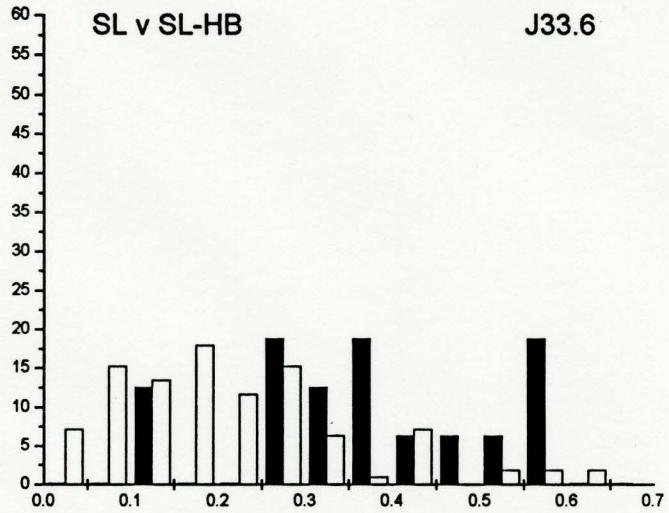
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7


SL v SL-HB
J33.6
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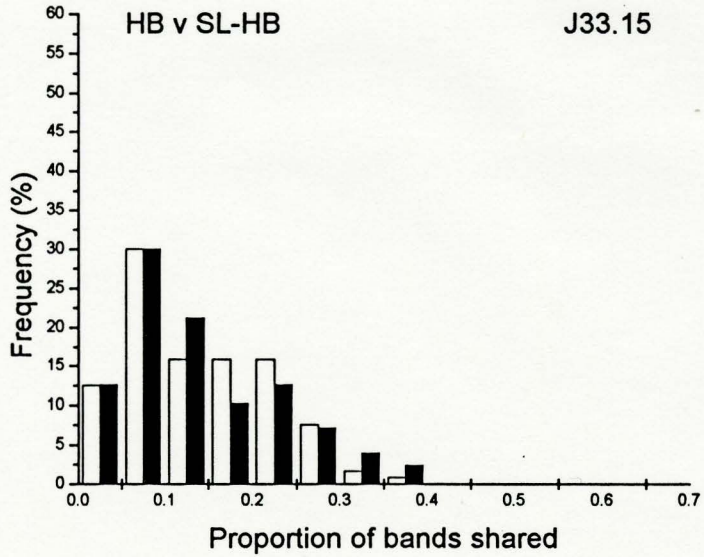
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7


HB v SL-HB
J33.15
Frequency (%)
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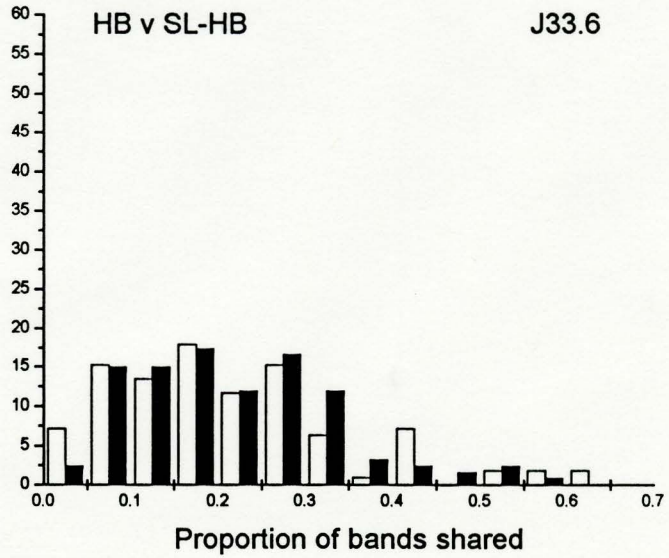
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Proportion of bands shared


HB v SL-HB
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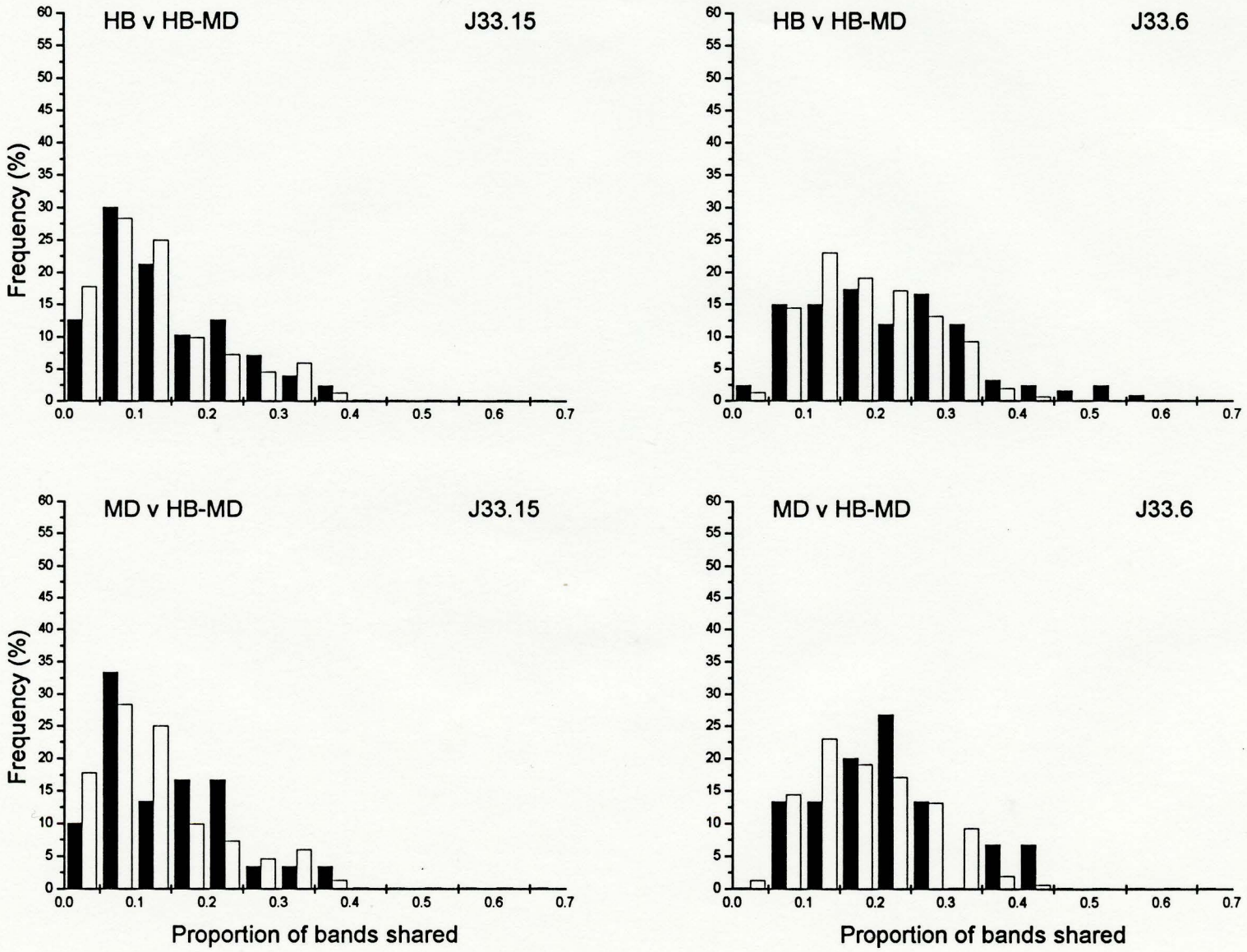
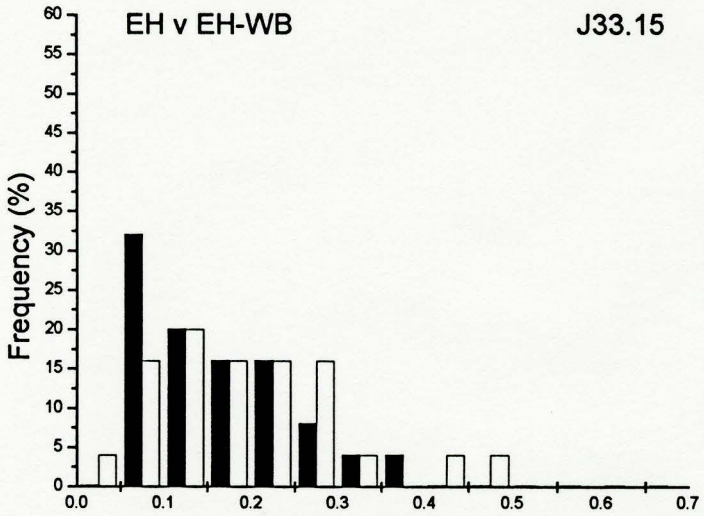
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Proportion of bands shared


HB v HB-MD
J33.15
HB v HB-MD
J33.6
MD v HB-MD
J33.15
MD v HB-MD
J33.6
Frequency (%)
Proportion of bands shared


EH v EH-WB
J33.15
Frequency (%)
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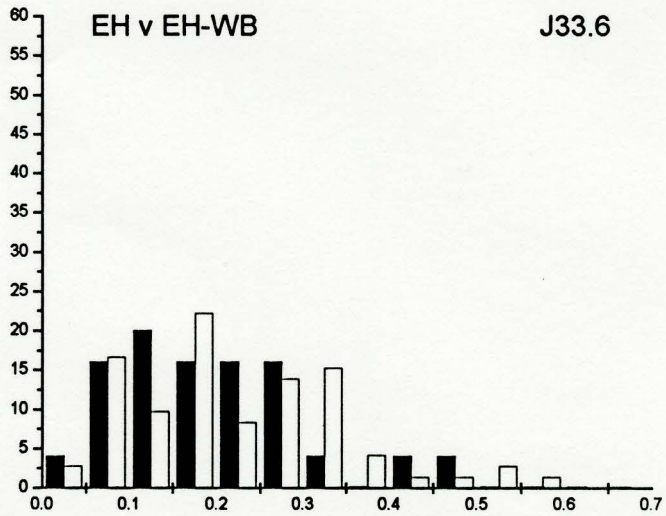
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7


EH v EH-WB
J33.6
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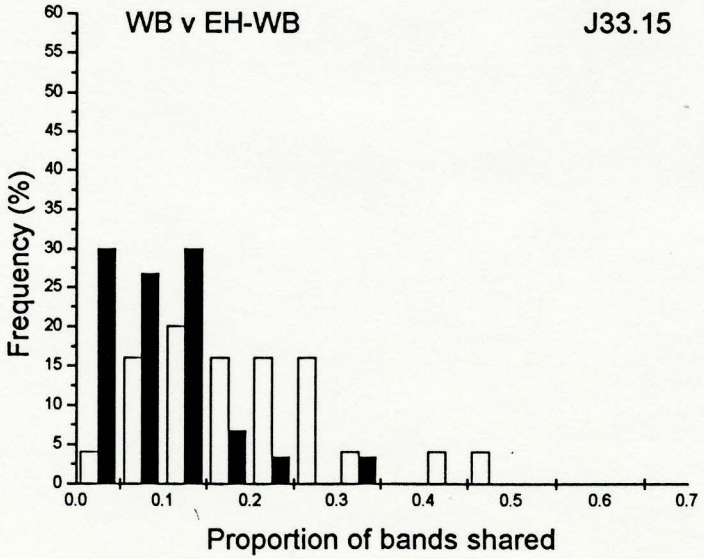
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7


WB v EH-WB
J33.15
Frequency (%)
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0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Proportion of bands shared

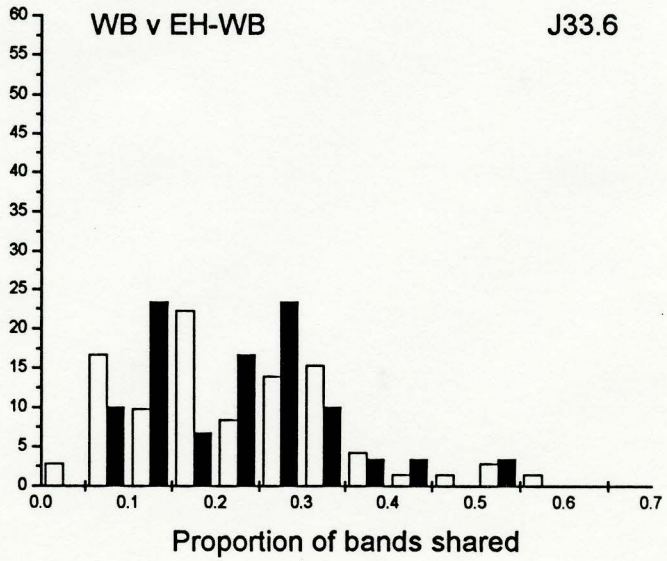
WB v EH-WB
J33.6
0 5 10 15 20 25 30 35 40 45 50 55 60
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Proportion of bands shared

TABLE 2.3. Mantel tests comparing bandsharing coefficient within (W) and between (B) populations of beluga whales in Canadian waters. Numbers represent probabilities associated with 9999 matrix permutations.

Macrogeographic Comparisons							
SL[1] v SL-HB	33.15	W > B	0.0001	HB v SL-HB	33.15	W > B	0.2688
	33.6	W > B	0.0024		33.6	W > B	0.2797
SL v SL-MD	33.15	W > B	0.0001	MD v SL-MD	33.15	W > B	0.2888
	33.6	W > B	0.0001		33.6	W > B	0.0001
HB v HB-MD	33.15	W > B	0.0110	MD v HB-MD	33.15	W > B	0.0214
	33.6	W > B	0.0001		33.6	W > B	0.0003
EH v EH-WB	33.15	W > B	0.0326	WB v EH-WB	33.15	B > W	0.0001
	33.6	B > W	0.0111		33.6	W > B	0.2667
St. Lawrence vs. Hudson Bay							
SL v SL-EH	33.15	W > B	0.0001	EH v SL-EH	33.15	W > B	0.0143
	33.6	W > B	0.0001		33.6	B > W	0.0676
SL v SL-WB	33.15	W > B	0.0001	WB v SL-WB	33.15	B > W	0.0001
	33.6	W > B	0.0001		33.6	W > B	0.0806
Mackenzie Delta vs Hudson Bay							
EH v EH-MD	33.15	W > B	0.0180	MD v EH-MD	33.15	W > B	0.2726
	33.6	W > B	0.0290		33.6	W > B	0.0001
WB v WB-MD	33.15	B > W	0.0002	MD v WB-MD	33.15	W > B	0.0077
	33.6	W > B	0.0014		33.6	W > B	0.0084

[1]Abbreviations: SL - St. Lawrence Estuary; HB - Hudson Bay; MD - Mackenzie Delta; EH - Eastern Hudson Bay; WB - Western Hudson Bay.

Lawrence comparisons is due to the influence of lower western and eastern Hudson Bay bandsharing for Jeffreys 33.15 and 33.6, respectively. A similar situation occurs for Mackenzie Delta Jeffreys 33.15 *D*-scores.

Mantel tests were also used to test the hypothesis that belugas on the eastern and western shores of the Hudson Bay interbreed to form a single genetic stock. There should be no substantial differences between intra- and interpopulation similarity estimates if populations interbreed. The results of these comparisons did not show a clear consensus (Table 2.3). Between-population bandsharing was significantly higher than within western-Hudson Bay for 33.15 and higher than within-eastern Hudson Bay for 33.6. Further, significance was not reached for higher within-population levels for the comparison involving western Hudson Bay for 33.6. These results could be taken as evidence of gene flow occurring between these populations. However, results for eastern Hudson Bay with 33.15 showed significantly lower between-population bandsharing. On this basis it was not possible to accept or reject the hypothesis of one genetic stock within Hudson Bay.

DISCUSSION

Typical average population bandsharing coefficients measured in natural populations of species not genetically impoverished range from 0.10 to 0.35 (Jeffreys *et al.* 1985b; Burke and Bruford 1987; Wetton *et al.* 1987; Birkhead *et al.* 1990; Gibbs *et al.* 1990; Pemberton *et al.* 1992), although higher values up to 0.60 or higher have been reported in some species, including those with no obvious population bottleneck history (e.g. pilot whales, Amos *et al.* 1991a,b; Hanotte *et al.* 1992). Most values for beluga whales (except for the St. Lawrence population) fell within the lower half of this range, indicating relatively high genetic diversity.

A previous fingerprinting study including the St. Lawrence and Mackenzie Delta populations found higher average bandsharing for both populations than reported here (Patenaude *et al.* 1994). The range of fingerprint band sizes scored was different for the two studies. Patenaude *et al.* (1994) scored bands as small as 2 kb, while the lower limit in this study was 3.5 kb. There are many bands smaller than 3.5 Kb, but the fingerprints lacked the band clarity and separation sufficient to score accurately. Bandsharing has been found to be higher for smaller fragment sizes (Jeffreys *et al.* 1985b; Bellamy *et al.* 1991), probably due to higher allele frequencies and comigration of unrelated fragments (Jeffreys *et al.* 1985a,b). The inclusion of such smaller fragments in the Patenaude *et al.* (1994) study

contributed to the higher estimates of bandsharing.

A second potential source of the differences is found in the relative amounts of band "crowding" seen in fingerprints in the two studies. DNA was run on fingerprint gels for longer distances in order to separate the bands in the scoring range to a greater degree than in the earlier study. The distribution of bands in fingerprints from the current study appear substantially more disperse. Therefore, there was probably a lower number of bands scored as identical which in fact differ slightly in length. This would result in lower bandsharing estimates than in more crowded fingerprints. Many of the bands observed previously were excluded because of comigration with more intense bands deemed to be different alleles (N. Patenaude, pers. comm.). This was not a problem in the current study, and few bands were excluded for this reason.

The average level of bandsharing calculated between fingerprints of western Hudson Bay beluga whales probed with Jeffreys 33.15 was surprisingly low (0.089). High genetic diversity is expected to occur in large populations (Wright 1969). Additionally, high diversity could be maintained if the western Hudson Bay population comprised a number of different wintering populations joining in summer. This is not likely in this case, as mitochondrial DNA studies have indicated that the western Hudson Bay population is genetically distinct from the closest population, which is in eastern Hudson Bay (Brennin 1992; Brennin *et al.*, in prep.). Further, the low values are not observed with Jeffreys 33.6, therefore this is likely not a population-based phenomenon.

An alternate explanation for low bandsharing involves the possibility of linkage of minisatellite loci. DNA fingerprinting is used with the assumption that

each band represents an independent, unlinked locus (Jeffreys *et al.* 1986; 1991). However, linked bands have been detected in mice (Jeffreys *et al.* 1987) birds (Hanotte *et al.* 1992) and dogs (Jeffreys and Morton 1987). Linkage would be expected to cause inaccurate estimates of genetic relatedness, inflating the variance (Lynch 1988).

Another uncertainty with fingerprints is the identity of alleles. The multilocus nature of fingerprints precludes the identification of specific alleles, because so many bands are seen, and because fragments of the same mobility are not necessarily isoallelic (Hill 1987). Various studies have used pedigree analysis to show that many of the bands detected represented alleles of only a few loci and that these alleles are represented by several cosegregating bands (Brock and White 1991; Hanotte *et al.* 1992; Galbraith *et al.* 1993). Brock and White (1991) identified as few as 2 to 5 loci, one of which was represented by as many as 19 cosegregating bands.

Allelism would, like linkage, increase the variance of bandsharing. Individuals sharing alleles with many bands in the scorable region would show extremely high bandsharing. However, animals which have different alleles or which share alleles comprised primarily of fragments smaller than the lower limit of scoring would yield very low *D*-scores. The nature of these alleles is unknown, but some may be long sequences with many repeats interspersed with restriction sites, generating multifragment alleles (Brock and White 1991). It is possible that the western Hudson Bay comparisons involved mainly individuals of the latter description. Since Jeffreys 33.6 detects an independent set of loci (Jeffreys *et al.* 1986, 1991), this pattern of allelism would not necessarily be seen for this probe.

It is not possible to determine the extent of allelism without pedigree information regarding segregation of fingerprinting bands (Burke 1989).

Mitochondrial DNA RFLP analysis indicates that eastern and western Hudson Bay populations are genetically distinct, and that such differentiation is maintained by philopatry of females to estuaries within the summering range of each population (Brennin 1992; Brennin *et al.*, in prep.). However, belugas from both coasts share a wintering ground (Finley *et al.* 1982; Richard *et al.* 1990) for a period encompassing the probable breeding season (Brodie 1971; Sergeant 1973). DNA fingerprinting was used to determine whether nuclear DNA gene flow is occurring via males breeding with females of other populations.

Comparisons of within-population to between-population bandsharing detected differences for only one probe in a single comparison. This may have been a consequence of the possible presence of small-fragment multiband alleles in western Hudson Bay individuals, which would not be scored in comparisons to other individuals, and might therefore underestimate similarity.

Three other comparisons within Hudson Bay which show non-significance of higher within-population variation or significantly higher between-population variation may be suggestive of a high level of gene flow between these populations. However, similar problems occurred in the use of Hudson Bay within-population variation in comparisons to other intra- and inter-population measures. This may reflect an inability to resolve patterns of population genetic diversity over the history of populations separated for periods of time longer than a few generations. Because minisatellite loci evolve so rapidly (e.g. 100-1000 times that of conventional allele variation), measures of their population variation

may be restricted to recent historic events (e.g. founder effects, bottlenecks, assortative mating) (Jeffreys *et al.* 1991). Variability may be so high at these loci that it is not possible to discriminate between variation within a population and that between populations. However, comparisons of mtDNA involving Mackenzie Delta and Hudson Bay belugas showed a consensus that these populations have differentiated since the recolonization of western Hudson Bay following the Wisconsin Ice Age, 7000 - 8000 years ago (Brennin 1992; Brennin *et al.*, in prep.). Recent microsatellite analysis has also detected differences between these Mackenzie Delta and western Hudson Bay belugas (Buchanan *et al.*, in prep.).

Comparisons of bandsharing within the St. Lawrence to that found between St. Lawrence and other belugas strongly indicate that this population is isolated from other Canadian stocks of belugas. The St. Lawrence population clearly showed lower genetic diversity than the other populations. This is consistent with the conclusion reached by Patenaude *et al.* (1994) that the St. Lawrence population has suffered a loss in genetic variation as a result of a severe depletion by intense hunting through to the mid-1900s (Reeves and Mitchell 1984; Pippard 1985). However, the level of bandsharing found here does not indicate an impact as severe as that concluded by Patenaude *et al.* (1994). Again, this may be due to difference in fingerprint analysis.

Genetic diversity in small, isolated populations is expected to become progressively reduced through drift (Wright 1969). as variants in the population become fixed at a rate higher than that for the generation of new length variants (e.g. Faulkes *et al.* 1990; Gilbert *et al.* 1990; Wayne *et al.* 1991). Similar or greater losses of variability have been reported for natural populations reduced in

numbers such as island populations of the California Channel Island fox (Gilbert *et al.* 1990), and populations of cheetahs, lions, marmots and seals which have suffered bottlenecks in the past (Gilbert *et al.* 1991; Menotti-Raymond and O'Brien 1990; Rassman *et al.* 1994; Kappe *et al.* 1995). Patenaude *et al.* (1994) also concluded that the reduced genetic diversity and low numbers may have compromised the capacity of the St. Lawrence beluga to recover due to possible inbreeding depression effects (Gilpin and Soulé 1986). The population size estimates of 350 to 500 (Reeves and Mitchell 1984; Pippard 1985; Sergeant 1986; Sergeant and Hoek 1990) imply that the effective size of the population is likely too low to neutralize the effects of drift and inbreeding (Franklin 1980; Frankel and Soulé 1981; Lande and Barrowclough 1987).

Conclusion

DNA fingerprinting was used in this study to detect population structuring of nuclear markers among populations of beluga whales in Canadian waters. This study confirmed the genetic isolation of the St. Lawrence stock, resulting in decreased genetic variation. Fingerprinting also indicated differences between Mackenzie Delta and Hudson Bay populations. Multilocus fingerprinting, however, could not provide sufficient resolution to evaluate the extent of genetic isolation of populations of beluga whales summering in eastern and western Hudson Bay.

It was not possible to determine whether possible linkage or allelism of detected fragments is responsible for lowered western Hudson Bay bandsharing estimates. This can not be decided without pedigree information from several

families of belugas (Burke 1989). Such information is not readily available because of the problems of sampling belugas in Arctic waters.

The use of other markers such as microsatellites, which allow identification of alleles, can give better insight into questions of gene flow and relationship of individuals in natural populations. Microsatellites have a high degree of variability due to rapid mutation rates (Edwards *et al.* 1992; Dallas *et al.* 1995; Amos *et al.* 1993), allowing their use in paternity and in more conventional measures of genetic distance and gene flow (e.g. Gotelli *et al.* 1992; Amos *et al.* 1993; Paetkau *et al.* 1995; Dallas *et al.* 1995). Beluga-specific microsatellites have been used to assess gene flow in other beluga populations (Buchanan *et al.*, in prep.) and to assign individuals to specific populations. Their application would therefore be useful in resolving the questions addressed in this chapter, as well as further questions regarding social structure within populations and smaller group units.

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APPENDIX. Data for samples of beluga whales collected and analyzed for mitochondrial DNA sequence variation. Tissues collected include heart (H), muscle (M), skin (S), kidney (K), liver (L), connective tissue (CT), and tendon (T).

DATA FOR BELUGA WHALES COLLECTED FROM THE NASTAPOKA RIVER

Sample Code #	Original Tag #	Collection Site	Date Collected	Sex	Colour	Tissue	Haplotype	Haplotype Group
DLE 001	DLN 87-01	Nastapoka River	07/13/87	F	n.a.	L	D01	A
DLE 002	DLN 87-02	Nastapoka River	07/13/87	M	n.a.	L	D01	A
DLE 007	DLN 87-07	Nastapoka River	08/06/87	M	n.a.	L	D01	A
DLE 008	DLN 87-08	Nastapoka River	08/06/87	M	n.a.	S	D01	A
DLE 009	DLN 87-09	Nastapoka River	08/06/87	F	n.a.	L	D01	A
DLE 010	DLN 87-10	Nastapoka River	08/06/87	F	n.a.	L	D01	A
DLE 011	DLN 87-11	Nastapoka River	08/06/87	F	n.a.	L	D01	A
DLE 012 *	DLN 87-11	Nastapoka River	08/06/87	n.a.	n.a.	L	D01	A
DLE 013	DLN 87-12	Nastapoka River	08/06/87	M	n.a.	L	D02	A
DLE 014	DLN 87-13	Nastapoka River	08/06/87	M	n.a.	L	D03	A
DLE 015	DLN 87-14	Nastapoka River	08/06/87	M	n.a.	L	D04	A
DLE 016	DLN 87-15	Nastapoka River	08/06/87	M	n.a.	L	D05	A
DLE 017	DLN 87-16	Nastapoka River	08/06/87	M	n.a.	L	D01	A
DLE 018	DLN 87-17	Nastapoka River	08/06/87	M	n.a.	L	D04	A
DLE 019	DLN 87-18	Nastapoka River	08/12/87	F	n.a.	L	D04	A
DLE 021	DLN 87-19	Nastapoka River	08/12/87	F	n.a.	L	D04	A
DLE 022	DLN 87-20	Nastapoka River	08/12/87	F	n.a.	L	D01	A
DLE 023	DLN 87-21	Nastapoka River	08/12/87	M	n.a.	L	D06	B
DLE 024	DLN 87-22	Nastapoka River	08/13/87	F	n.a.	L	D07	B
DLE 025	DLN 87-23	Nastapoka River	08/13/87	M	n.a.	L	D03	A
DLE 026	DLN 87-24	Nastapoka River	08/17/87	F	n.a.	L	D04	A
DLE 027	DLN 87-25	Nastapoka River	08/17/87	F	n.a.	L	D04	A
DLE 028	DLN 87-26	Nastapoka River	08/17/87	F	n.a.	L	D01	A
DLE 029	DLN 87-27	Nastapoka River	08/17/87	F	n.a.	L	D01	A
DLE 030 *	DLN 87-28	Nastapoka River	08/20/87	M	n.a.	L	D01	A
DLE 031	DLN 87-29	Nastapoka River	08/20/87	F	n.a.	L	D01	A

DATA FOR BELUGA WHALES COLLECTED FROM THE NASTAPOKA RIVER (continued)

Sample Code #	Original Tag #	Collection Site	Date Collected	Sex	Colour	Tissue	Haplotype	Haplotype Group
DLE 032 *	DLN 87-30	Nastapoka River	08/20/87	M	n.a.	L	D01	A
DLE 033	DLN 87-31	Nastapoka River	08/20/87	F	n.a.	L	D01	A
DLE 034	DLN 87-32	Nastapoka River	08/20/87	F	n.a.	L	D01	A
DLE 035	DLN 87-33	Nastapoka River	08/21/87	F	n.a.	L	D01	A
DLE 036	DLN 87-34	Nastapoka River	08/21/87	M	n.a.	L	D01	A
DLE 037	DLN 87-35	Nastapoka River	08/21/87	M	n.a.	L	D04	A
DLE 038	DLN 87-36	Nastapoka River	08/21/87	F	n.a.	L	D01	A
DLE 039	DLN 87-37	Nastapoka River	08/21/87	M	n.a.	L	D04	A
DLE 041	DLN 87-39	Nastapoka River	08/24/87	F	n.a.	L	D01	A
DLE 042	DLN 87-40	Nastapoka River	08/24/87	F	n.a.	L	D01	A
DLE 043	DLN 87-41	Nastapoka River	08/24/87	M	n.a.	L	D07	B
DLE 044	DLN 87-42	Nastapoka River	08/26/87	M	n.a.	L	D03	A
DLE 045	DLN 87-43	Nastapoka River	08/26/87	F	n.a.	L	D01	A
DLE 426	n.a.	Nastapoka River	08/18/94	F	G	S	D08	B
DLE 427	n.a.	Nastapoka River	08/18/94	F	W	S	D03	A
DLE 467	IN-94-01	Nastapoka River	08/15/94	F	G	H	D01	A
DLE 468	IN-94-02	Nastapoka River	08/16/94	M	W	H	D04	A
DLE 469	IN-94-03	Nastapoka River	08/16/94	F	G	H	D01	A
DLE 470	IN-94-05	Nastapoka River	08/16/94	F	W	H	D01	A
DLE 471	IN-94-06	Nastapoka River	08/16/94	M	G	H	D04	A
DLE 472	IN-94-07	Nastapoka River	08/16/94	F	W	H	D04	A
DLE 473	IN-94-08	Nastapoka River	08/16/94	M	G	H	D06	B
DLE 474	UM-94-10	Nastapoka River	08/16/94	F	G	M	D04	A
DLE 475	UM-94-13	Nastapoka River	08/16/94	F	G	H	D06	B
DLE 476	UM-94-15	Nastapoka River	08/16/94	F	G	H	D04	A

DATA FOR BELUGA WHALES COLLECTED FROM THE LITTLE WHALE AND GREAT WHALE RIVERS

Sample Code #	Original Tag #	Collection Site	Date Collected	Sex	Colour	Tissue	Haplotype	Haplotype Group
DLE 379	92-LW-01	Little Whale River	08/09/92	F	W	S	D05	A
DLE 380	92-LW-02	Little Whale River	08/09/92	F	G	H	D02	A
DLE 381	92-LW-03	Little Whale River	08/10/92	F	W	S	D02	A
DLE 392	93-UMIUJAQ-01	South of LWR	07/17/93	M	W	H,M	D08	B
DLE 393	93-UMIUJAQ-02	South of LWR	07/17/93	M	W	H	D09	B
DLE 422	n.a.	Little Whale River	08/12/93	F	W	S	D08	B
DLE 423 *	n.a.	Little Whale River	08/12/93	F	G	S	D08	B
DLE 424	n.a.	Little Whale River	08/15/93	F	W	S	D12	A
DLE 425 *	n.a.	Little Whale River	08/15/93	M	G	S	D12	A
DLE 430	93-KUUJJUARAAPIK-05	Little Whale River	07/29/93	F	W	H	D05	A
DLE 428	93-KUUJJUARAAPIK-01	Great Whale River	07/02/02	M	W	H	D06	A
DLE 429	93-KUUJJUARAAPIK-02	Great Whale River	07/02/93	M	W	H	D06	A
DLE 460	DL-GW-03	Great Whale River	07/25/94	F	G	H	D05	A
DLE 461	DL-GW-07	Great Whale River	07/25/94	F	W	H	D04	A
DLE 462	DL-GW-08	Great Whale River	n.a.	F	W	M	D16	B
DLE 463	DL-GW-09	Great Whale River	07/25/94	F	W	H	D17	B
DLE 464	DL-GW-12	Great Whale River	07/25/94	F	G	M	D02	A
DLE 465	DL-GW-13	Great Whale River	07/25/94	F	D	H	D01	A
DLE 466	DL-GW-15	Great Whale River	07/25/94	F	W	H	D05	A

DATA FOR BELUGA WHALES COLLECTED FROM SOUTHERN HUDSON STRAIT

Sample Code #	Original Tag #	Collection Site	Date Collected	Sex	Colour	Tissue	Haplotype	Haplotype Group
DLE 399	93-QUAQTUQ-06	Augaktuk	07/02/93	F	D	H	D08	B
DLE 400	93-QUAQTUQ-08	n.a	06/22/93	F	D	H	D06	B
DLE 401	93-QUAQTUQ-01	Augaktuk	07/02/93	F	W	H	D08	B
DLE 402	93-QUAQTUQ-15	Naujaak	06/23/93	M	W	H,M	D08	B
DLE 403	93-QUAQTUQ-16	Augatumi	06/19/93	M	G	CT	D01	A
DLE 404	93-QUAQTUQ-17	Augatumi	07/12/93	M	W	T	D10	B
DLE 405	93-QUAQTUQ-19	Naujaak	06/23/93	M	W	H	D08	B
DLE 406	93-QUAQTUQ-20	Naujaak	06/16/93	F	W	H	D02	A
DLE 407	93-QUAQTUQ-21	Imilimi	n.a	F	G	H,M	D08	B
DLE 408	93-QUAQTUQ-23	Augatumi	07/02/93	M	W	H	D08	B
DLE 409	93-QUAQTUQ-26	Naujaak	06/23/93	M	W	H	D05	A
DLE 410	93-QUAQTUQ-29	Naujaak	06/16/93	F	W	H	D05	A
DLE 411	93-QUAQTUQ-30	Naujaak	06/23/93	M	G	H	D06	B
DLE 412	93-SALLUIT-01	Salluit	n.a.	M	W	H	D08	B
DLE 413	93-SALLUIT-02	Salluit	n.a.	M	W	H	D11	B
DLE 414	93-SALLUIT-03	Salluit	n.a.	F	W	H	D08	B
DLE 415	93-SALLUIT-04	Salluit	n.a.	M	G	H	D08	B
DLE 416	93-SALLUIT-05	Salluit	n.a.	F	G	H	D08	B
DLE 417	93-SALLUIT-06	Salluit	n.a.	M	W	H	D08	B
DLE 418	93-SALLUIT-08	Salluit	n.a.	F	D	H	D08	B
DLE 419	93-KANGIQSUJUAQ-05	Wakeham Bay	07/29/93	M	G	H	D08	B

DATA FOR BELUGA WHALES COLLECTED FROM UNGAVA BAY

Sample Code #	Original Tag #	Collection Site	Date Collected	Sex	Colour	Tissue	Haplotype	Haplotype Group
DLE 394	93-KANGIRSUK-01	Qinituut	06/27/93	F	G	H	D08	B
DLE 395	93-KANGIRSUK-06	Payne Bay	07/17/93	F	D	H	D01	A
DLE 396	93-KANGIRSUK-09	Payne Bay	07/17/93	M	W	H	D06	B
DLE 397	93-KANGIRSUK-12	Qinituut	06/28/93	M	W	H,M	D08	B
DLE 398	93-KANGIRSUK-14	Qinituut	06/27/93	F	G	H	D06	B
DLE 459	KG-94-10	Kangirsuk	1994	F	D	H	D11	B
DLE 420	93-KUJJUAQ-06	Kisavik	08/08/93	M	W	S	D10	B
DLE 421	93-KUJJUAQ-10	Unguinav	08/01/93	F	G	S	D08	B
DLE 431	93-KANGIQSUALUJJUAQ-01	off the George R.	n.a.	M	W	H	D13	B
DLE 457	TA-94-08	5837N6947W	8/16/94	F	W	S	D08	B
DLE 458	TA-94-10	5837N6947W	8/16/94	F	D	S	D11	B

DATA FOR BELUGA WHALES COLLECTED FROM WAKEHAM BAY IN AUTUMN

Sample Code #	Original Tag #	Collection Site	Date Collected	Sex	Colour	Tissue	Haplotype	Haplotype Group
DLE 117	83-DLWB-18	Wakeham Bay	10/93	F	n.a.	K	D14	B
DLE 118	83-DLWB-17	Wakeham Bay	10/93	M	n.a.	K	D15	A
DLE 119	83-DLWB-16	Wakeham Bay	10/93	F	n.a.	K	D08	B
DLE 120	83-DLWB-15	Wakeham Bay	10/93	M	n.a.	K	D08	B
DLE 121	83-DLWB-14	Wakeham Bay	10/93	F	n.a.	K	D15	A
DLE 122	83-DLWB-12	Wakeham Bay	10/93	M	n.a.	H	D03	A
DLE 123	83-DLWB-11	Wakeham Bay	10/93	M	n.a.	K	D04	A
DLE 124	83-DLWB-10	Wakeham Bay	10/93	M	n.a.	M	D02	A
DLE 125	83-DLWB-09	Wakeham Bay	10/93	F	n.a.	K	D13	B
DLE 126	83-DLWB-08	Wakeham Bay	10/93	M	n.a.	K	D02	A
DLE 127	83-DLWB-07	Wakeham Bay	10/93	F	n.a.	K	D05	A
DLE 128	83-DLWB-06	Wakeham Bay	10/93	F	n.a.	L	D08	B
DLE 129	83-DLWB-05	Wakeham Bay	10/93	F	n.a.	L	D08	B
DLE 130	83-DLWB-03	Wakeham Bay	10/93	M	n.a.	S	D05	A
DLE 131	83-DLWB-02	Wakeham Bay	10/93	F	n.a.	K	D06	B
DLE 447	WA-94-10	Kangisuapik	10/17/94	M	W	H	D08	B
DLE 448	WA-94-17	Kangisuapik	10/17/94	M	W	H	D15	A
DLE 449	WA-94-20	Kangisuapik	10/17/94	M	W	H	D09	B
DLE 450	WA-94-21	Kangisuapik	10/17/94	M	W	H	D08	B
DLE 451	WA-94-01	Itiviani	10/19/94	M	W	H	D08	B
DLE 452 +	WA-94-05	Itiviani	10/19/94	M	W	H	n.a.	n.a.
DLE 453	WA-94-09	Itiviani	10/19/94	F	G	H	D08	B
DLE 454	WA-94-13	Itiviani	10/19/94	M	G	H	D09	B
DLE 455	WA-94-18	Itiviani	10/19/94	M	G	H	D08	B
DLE 456	WA-94-29	Itiviani	10/19/94	F	W	H	D08	B