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USE OF DNA MICROSATELLITES IN BELUGA WHALE (*Delphinapterus leucas*) POPULATION GENETICS

by

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The potential of nuclear DNA microsatellite analysis for use as a marker for population genetic studies of beluga whales (*Delphinapterus leucas*) was tested. One microsatellite locus was amplified in beluga samples from five different locations using primers developed for the long-finned pilot whale (*Globicephala melas*).

The distribution of genotypes among whales sampled at each location was in agreement with the Castle-Hardy-Weinberg equilibrium. The test could not be performed on the St. Lawrence whales where only two alleles were found distributed as 17 homozygotes and 1 heterozygote. Examination of the five alleles amplified revealed significant allele frequency differences among beluga from Alaska, Mackenzie Delta, Nastapoka River, and St. Lawrence River. Comparison of the allele frequencies among males and females where there were sufficient samples (Alaska, Mackenzie Delta, and Nastapoka River) showed no significant differences. Significant allele frequency differences were not found among whales sampled at Pt. Lay, Alaska and whales sampled at Norton Sound, Alaska. This result, however, may be a reflection of the small sample size (n=13) from Norton Sound.

Future studies of beluga whale population genetics and stock identification should focus on the analysis of more microsatellite loci to increase the resolving power of the technique.

Key words: beluga; *Delphinapterus leucas*; population genetics; nuclear DNA; microsatellites.

RÉSUMÉ

Maiers, L.D., M.K. Friesen, A.V. Wiens, and J.W. Clayton. 1996. Use of DNA microsatellites in beluga whale (*Delphinapterus leucas*) population genetics. Can. Tech. Rep. Fish. Aquat. Sci. 2115: iv + 17p.

Nous avons testé le potentiel de l'analyse des microsatellites de l'ADN nucléaire pour établir des marqueurs dans les études de génétique des populations de béluga (*Delphinapterus leucas*). Un locus de microsatellite a été amplifié dans des échantillons de bélugas provenant de cinq endroits différents à l'aide d'amorces développées pour le globicéphale commun (*Globicephala melas*).

La distribution des génotypes parmi les cétacés échantillonnés à chaque endroit concordait avec l'équilibre de Castle-Hardy-Weinberg. Le test n'a pas pu porter sur les

bélugas du Saint-Laurent, chez qui on n'a trouvé que deux allèles, répartis en 17 formes homozygotes et une forme hétérozygote. L'examen des cinq allèles amplifiés a révélé des différences significatives dans la fréquence allélique entre les bélugas de l'Alaska, du delta du Mackenzie, de la Nastapoka et du Saint-Laurent. La comparaison des fréquences alléliques entre les mâles et les femelles quand les échantillons étaient suffisants (Alaska, delta du Mackenzie et Nastapoka) n'a pas révélé de différences significatives. Il n'y avait pas de différences significatives entre les cétacés échantillonnés à la pointe Lay (Alaska) et dans le Norton Sound (Alaska). Ce dernier résultat pourrait toutefois refléter la faible taille de l'échantillon (n = 13) du Norton Sound.

Les travaux futurs sur la génétique des populations et l'identification des stocks de bélugas devraient mettre l'accent sur l'analyse d'un nombre plus grand de locus de microsatellites pour accroître le pouvoir de résolution de cette technique.

Mots clés : béluga; *Delphinapterus leucas*; génétique des populations; ADN nucléaire; microsatellites.

INTRODUCTION

The beluga whale, *Delphinapterus leucas*, is widely distributed in arctic and subarctic waters of North America . Harvesting of these whales is a major cultural feature for many northern Canadian aboriginal communities. Most beluga migrate between poorly defined wintering areas in broken ice cover and summering areas in estuarine and other nearshore waters (Cosens and Dueck 1990). The numbers of some summering concentrations of beluga whales are depleted. The non-migratory St. Lawrence River beluga, for example, have been assigned an endangered status by COSEWIC (Committee On the Status of Endangered Wildlife in Canada) (Campbell 1994). Effective management, in consideration of the aboriginal cultural imperative of a sustainable harvest, requires a more thorough understanding of stock structure. Nuclear DNA markers would provide information on the breeding strategy of the beluga and contribute to an understanding of the genetic discreteness of the various summer concentrations. Microsatellite analysis, or the examination of simple repeat sequence length polymorphisms in nuclear DNA, is a technique which may be used to reveal this information.

Microsatellites have been employed in a wide range of applications such as the construction of genetic maps (e.g. Dietrich et al. 1992; Rohrer et al. 1994; Weissenbach et al. 1992), the determination of evolutionary relationships (e.g. Buchanan et al. 1994), human disease research (e.g. Carey et al. 1994; Wooster et al. 1994) and forensic investigations (e.g. Hagelberg et al. 1991; Debenham 1994). They also have been used in intraspecific genetic studies (Paetkau and Strobeck 1994). Microsatellites have a relatively rapid mutation rate that makes them more useful than sequencing analysis of nucleotide substitutions for constructing phylogenetic trees (Dietrich et al. 1992) and for the analysis of genetic variation within a species (Buchanan pers. comm.). It has been shown that microsatellites may have the ability to trace gene flow among populations (Dietrich et al. 1992). The mutation rate of the number of repeat units is fast enough to allow polymorphism to accumulate within populations, but not frequent enough for differences to occur between successive generations within one subpopulation (Tautz 1989).

The usefulness of microsatellites for the intra-specific study of whale populations has already been shown (Schlötterer et al. 1991; Amos et al. 1993; Valsecchi et al. 1993). Microsatellite sequences are usually embedded within unique stretches of DNA at a frequency of one simple sequence repeat series every 10 kb of DNA (Tautz 1989). The unique sequences flanking each stretch of repeats are therefore a source of primers for polymerase chain reaction (PCR) amplification of the microsatellite. These primer sequences may be found by using sequences already

stored in sequence databases or by screening genomic libraries with probes of simple repeat sequences (Buchanan 1993). Once found, positive clones may be sequenced to reveal the primers. Of even greater benefit is the fact that primer-complementary sequences for simple sequence loci are often conserved among whale species (Schlötterer et al. 1991; Valsecchi et al. 1993).

The purpose of this study was to determine the ability of microsatellite analysis to detect genetic variability in beluga whale populations from various geographic locations. Primers developed for the 464/465 microsatellite locus in the pilot whale (Schlötterer et al. 1991) were tested for their ability to amplify the microsatellite in beluga whales. The amplified microsatellite was then tested for its potential to examine the genetic relationship among beluga from four North American locations: Alaska, Mackenzie Delta, Nastapoka River (eastern Hudson Bay), and St. Lawrence River. At a finer level of resolution, Point Lay, Alaska and Norton Sound, Alaska beluga were also compared to examine groups in close geographic proximity.

MATERIALS AND METHODS

Beluga whale sample collection and preservation

Two hundred and fourteen beluga whale tissue samples were collected from five Arctic and subarctic locations across North America (Fig. 1): Norton Sound, Alaska (n = 13), Point Lay, Alaska (n=50), Mackenzie Delta (n = 108), Nastapoka River (n = 26), and St. Lawrence River (n = 17). At all locations except the St. Lawrence River, samples were from animals taken during aboriginal subsistence hunts. St. Lawrence River samples were collected by Dr. Pierre Béland from necropsied whales that had been found dead in the river or washed onto shore.

Two methods of sample preservation were used. In some cases, approximately 100 g samples of muscle, liver, kidney, and heart tissue were frozen in the field and shipped frozen to the laboratory. The second and preferred method was to store approximately 1.0 - 5.0 g of skin in a salt-saturated 20% dimethyl sulphoxide (DMSO) solution containing 0.5 M EDTA (Amos and Hoelzel 1991). The pickled skin samples do not need to be frozen which is often very difficult in remote collection locations.

DNA extraction

DNA was extracted from tissue samples using methods adapted from Helbig et al. (1989) and Sambrook et al. (1989). Approximately 3.5 mL of lysis buffer (commercially prepared by Applied Biosystems) was mixed with approximately 0.5g of preserved tissue sample in a mortar. The tissue was then cut into very fine pieces using dissecting scissors and the entire mixture frozen with liquid nitrogen. It was pulverized into a fine powder using a pestle or

shaved with a scalpel. The powder was scraped into a disposable polypropylene tube, capped and incubated in a 37°C water bath. After 16 - 18 hours, 50 µL of a 20 mg/mL solution of proteinase K (Gibco/BRL) were added to the tube and incubated for 24 - 72 hours at 37°C. Further additions of proteinase K were made and the incubation was extended up to several weeks, if required, until the tissue sample was completely dissolved and the liquid became clear.

Once the tissue was digested, total cellular DNA in the lysis buffer/proteinase solution was extracted using an equal volume of a solution containing phenol:chloroform:isoamyl alcohol (25:24:1). The aqueous and organic phases were separated using centrifugation at high speed in an I.E.C. clinical centrifuge for 10 min. at room temperature. The aqueous layer was transferred to a clean tube and an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) again was added and the mixture emulsified. Centrifugation was repeated using the same conditions. The addition of phenol:chloroform:isoamyl alcohol and centrifugation was repeated until there was no visible layer at the interface. At this point, the aqueous layer was transferred to a clean tube and an equal volume of chloroform:isoamyl alcohol (24:1) added, the mixture emulsified, and centrifuged for 10 min. at high speed in an I.E.C. clinical centrifuge at room temperature. The aqueous layer was then added to a clean COREX glass tube, avoiding the transfer of any chloroform and residual solid material.

The DNA was precipitated by adding 0.1 volume of 3.0 M sodium acetate or ammonium acetate and 1 volume of isopropyl alcohol. The mixture was swirled and in most cases the DNA visibly precipitated immediately. If no precipitate was visible, the sample was incubated in a refrigerator (4 -8°C) for 15 - 30 minutes. DNA was then pelleted by centrifugation at 10,000 rpm for 10 min. at 4°C. The supernatant was removed and the pellet washed with 5.0 mL of 70% ethanol. The DNA was again recovered by centrifugation at 10,000 rpm for 2 min. at 4°C. Washing and centrifugation were repeated and the ethanol removed. Between 400 and 1000 µL of sterile TE buffer (Tris-EDTA, pH 8.0) (Sambrook et al. 1989), depending on the size of the DNA pellet, were added to each tube to resuspend the DNA. No attempt was made to physically resuspend the pellet in the buffer to avoid breaking or shearing the DNA. The tubes were stored overnight in the refrigerator and the pellet and buffer were then gently mixed with a pipette to resuspend the DNA. The DNA was transferred to a 1.5 mL Eppendorf tube, sealed, and stored at -20°C.

Radioactive labelling of microsatellite primer

A 20 µM solution of the *G. melas* 464-1 primer (synthesized at the University of Calgary Oligonucleotide Synthesis Laboratory) was end-labelled with either γ^{32}P-[dATP] or γ^{33}P-[dATP] (EasyTide™ radioisotope, NEN-DuPont) using a T4 polynucleotide kinase reaction (Sambrook et al. 1989). A reaction mixture of 26 µL sterile distilled water, 2.0 µL

of 20 mM *G. melas* 464-1 primer stock (final concentration approximately 1 mM), 4.0 µL 10X T4 polynucleotide kinase buffer (Boehringer-Mannheim), 6.0 µL radioactively labelled nucleotide, and 20 units of T4 polynucleotide kinase (Boehringer-Mannheim) was incubated at 37°C for 30 minutes. The reaction was stopped by heating at 65°C for 10 minutes.

PCR amplification of the microsatellite in beluga whales

A PCR reaction cocktail was made using 10.9 µL sterile distilled water, 1.2 µL of a 2.5mM stock solution of each dNTP, 1.0 µL 25 mM $MgCl_2$, 2.2 µL 10X *Taq* polymerase buffer, 2.5 µL of each 2 µM *G. melas* 464-1 and *G. melas* 464-2 microsatellite primer (unlabelled), and 1 unit of Taq DNA polymerase (Boehringer-Mannheim). To this reaction mixture was added approximately 100 ng of beluga genomic DNA and 0.5 µL of the 1µM radioactively labelled *G. melas* 464-1 primer. Approximately 10 µL of mineral oil was added to overlay the mixture to prevent evaporation of the reaction mixture during thermal cycling.

Amplification was performed in a Perkin-Elmer thermal cycler and began with an initial DNA denaturation at 94°C for 4 minutes, followed by 30, 3-step cycles of 30 sec. at 94°C, 1 min. at 58°C, and 30 sec. at 72°C. A final extension was run at 72°C for 4 minutes.

Polyacrylamide gel electrophoresis of microsatellites

Microsatellite amplification products were separated by electrophoresis in a polyacrylamide sequencing gel. Six microlitres of amplification reaction mixture were combined with 5 µL of stop solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF) and heated at 80°C for 5 - 10 minutes then loaded onto an 8% polyacrylamide gel that had been covered with 1X TBE buffer and pre-warmed to 45 - 50°C. Electrophoresis was run at 45 - 50 watts for 1.5 hours. Upon completion of electrophoresis, the gel was transferred to Whatman 3MM filter paper, covered with plastic wrap, and dried for 1 - 2 hrs. in a BioRad gel dryer with vacuum. The dried gel was covered with a fresh piece of plastic wrap (for ^{32}P label), placed into an autoradiography cassette, and Kodak XAR x-ray film placed against the gel. Films were exposed at -70°C for 14 - 36 hrs. with intensifying screen for ^{32}P or 36 - 120 hrs. at room temperature for ^{33}P. X-ray film was developed using standard techniques (Sambrook et al. 1989).

Sizing of microsatellites

Amplification products from a cycle sequencing reaction of M13mp18 were used to provide a standard to estimate the sizes of the different microsatellite allele patterns observed in this study. Control M13mp18 DNA and M13 universal sequencing primer (United States Biochemical-Amersham

Canada) were used in a Sequitherm cycle sequencing kit reaction (Cedarlane). The M13 sequencing primer was end-labelled using γ^{32}P-[dATP]. A sequencing reaction was made containing 5.0 µL sterile distilled water, 2.5 µL 10X enzyme buffer, 1 µg M13 DNA, 2 units DNA polymerase, and 1.0 µL of 1 mM end-labelled primer. Four tubes were designated A, C, G, and T and had 2.0 µL of the appropriate dideoxy-termination mix added. On ice, 4.0 µL of the sequencing reaction mix was added to each of the termination mix tubes. This mixture was overlaid with approximately 10 µL of mineral oil and heated in a Perkin-Elmer thermocycler for 5 min. at 95°C. The reaction was then subjected to cycles of 30 sec. at 95°C, 30 sec. at 50°C, and 1 min. at 70°C for 30 cycles. The reactions were stopped with the addition of 5 µL stop solution, heated at 80°C for 5 min., and then 6.0 µL aliquots of each reaction mixture were loaded onto the sequencing gel along with the microsatellites.

The primer sequence and the M13 sequence, provided by the United States Biochemical Company, were used to count the cumulative number of nucleotides in the sequence. Microsatellite size (nucleotide units) was determined from the position of the bands relative to the M13 sequence.

Sequencing bands and microsatellite bands were evaluated qualitatively by eye on a light table. Pairs of microsatellite bands were determined to be heterozygous if the lower, more anodal, band was of equal or greater intensity relative to the upper band. Once microsatellite allele size patterns were established, samples displaying the various sizes were run on every gel along with unknowns in order to provide references for the determination of genotype and size. In addition, the inclusion of these samples during every PCR run and on each electrophoresis gel demonstrated the reproducibility of the method. A fin whale (*Balaenoptera physalus*) sample was used as a positive control in every set of amplifications and also served as an orientation marker and a size marker of 145 nucleotides.

Analysis of microsatellite data

The agreement between the observed and the expected numbers of genotypes calculated according to the Castle-Hardy-Weinberg equilibrium was evaluated for whales from each location using the probability test and the heterozygote deficiency test in GENEPOP (ver. 2.0) Population Genetics software (Raymond and Rousset 1995). Both tests use a null hypothesis that the population is a randomly mating population with a random union of gametes. The difference between the two tests is in the construction of the rejection zone. The heterozygote deficiency test is the more powerful of the two for detecting an excess of homozygosity (which may indicate the presence of null alleles) (Rousset and Raymond 1995).

The null hypothesis that samples from separate locations represent subsamples of a

single interbreeding population was tested by calculating exact probabilities from pairwise contingency table tests of total numbers of alleles for all whales at each sampling location. In addition, a comparison of alleles in males and females at sites with available whale sex information was performed also using pairwise contingency table tests. Row and column independence was tested using the Pearson's Chi-squared and the Fisher's exact test. These tests were performed using the StatXact-Turbo computer software package (CYTEL Software Corporation, Cambridge, Massachusetts) which allows for tests of significance on data sets that are small and/or skewed. Nominal rejection level was $p = 0.05$, but for location comparisons it was reduced to 0.01 to adjust for multiple comparisons (Glantz 1992).

RESULTS AND DISCUSSION

Primers designed for the long-finned pilot whale 464/465 microsatellite locus successfully amplified a microsatellite locus in the beluga whale. Seven phenotypes corresponding to seven genotypes representing five different alleles were observed from the microsatellite amplification products of the beluga samples from the geographic locations examined (Table 1 and Fig. 2). This was comparable to the eight alleles found in the pilot whale (Amos et al. 1993). The products of beluga DNA amplified by the 464/465 pilot whale primers were found to range from 130 to 138 nucleotides in length.

The M13 sequencing reaction used to estimate the size of the alleles also confirmed that the major bands in different phenotypes differ by increments of two nucleotides, as is to be expected for a GT dinucleotide repeat unit (Schlötterer et al. 1991). The shadow or "slippage" bands evident in the autoradiograph of the microsatellite alleles are the result of *Taq* polymerase errors during the amplification of the microsatellite (Hoelzel and Bancroft 1992). During amplification, the polymerase may 'slip' causing it to miss the incorporation of one or two of the repeat units. This results in the production of a microsatellite product two or four etc... nucleotides shorter in length than the actual microsatellite template. These products usually appear as much fainter bands than the true product on the autoradiograph as they are produced in lesser quantity. However, these bands often make it difficult to correctly identify heterozygous individuals.

In microsatellite studies "null alleles" may occur for several reasons (Bruford et al. 1992). One source of a null allele is a polymorphism in the sequence which causes the failure of the amplification. This may be caused, for example, by a base change in a site at or near the primer binding site. In a homozygote, this type of null allele may cause a blank lane on the autoradiogram which is incorrectly interpreted as a technical failure rather than an

actual discrete allele. In a heterozygote, the most probable situation for rare null alleles, an allele of this type would probably lead to the interpretation of the phenotype as a homozygote for the other normal allele. The data set for a population with null allele(s) may therefore appear to exhibit an excess of homozygotes when compared to the expected values predicted by Castle-Hardy-Weinberg equilibrium. The data produced for the beluga using the pilot whale 464/465 microsatellite primers did not disagree with Castle-Hardy-Weinberg equilibrium (Table 2), indicating there few or no null alleles. However, the testing of agreement to the Castle-Hardy-Weinberg equilibrium, even with the advanced methods available in GENEPOP, is not particularly sensitive and it would be desirable to test for null alleles with family pedigree data whenever possible.

Before results may be compared among locations, it should be determined that there is no difference in the distribution of the numbers of alleles between males and females at each location. In the Nastapoka River and Point Lay, Alaska whales, no significant differences were found (p=0.0882 and p=0.0486 respectively). Application of Fisher's exact test (Table 3) to numbers of alleles in male and female samples from the Mackenzie Delta resulted in an exact p-value of 0.0486. When rounded to 0.05, this value may be considered as not significantly different when the usual rejection level of <0.05 is considered. This value may be due to the large sampling area covered in the Mackenzie Delta, resulting in samples being taken from subgroups of whales which were not sampled equally with respect to sex. However, to clarify that the distribution of alleles is homogenous among males and females in this population, more microsatellite loci should be tested.

The combined male and female data set for all locations (Table 1) was in agreement with Castle-Hardy-Weinberg equilibrium. Agreement of the observed data set with the calculated Castle-Hardy-Weinberg equilibrium indicates that for each location, the whales used in this study are representative of randomly mating, homogenous populations.

The purpose of the pilot whale 464/465 microsatellite locus analysis was to determine if the method of microsatellite analysis has potential as a nuclear marker method for the differentiation of beluga from various geographic regions. To test the method at different levels of resolution, beluga samples from widely separated locations and adjacent locations were tested. At a coarse level of resolution, western arctic beluga (Alaska and Mackenzie Delta) were compared to eastern arctic/subarctic beluga (Nastapoka River and St. Lawrence River). These groups are separated by distance and land/ice barriers. Allele distributions between these groups were found to be significantly different from one another (Table 4), indicating that whales

sampled at these locations do not belong to a single, interbreeding population.

Whales from the St. Lawrence River were almost homozygous for one allele (only one heterozygote among 17 individuals), supporting the hypothesis that this is an isolated population with reduced genetic variability (Patenaude et al. 1994). However, caution should be used in the interpretation of results from only one genetic locus.

At a finer level of resolution, beluga microsatellite allele distributions from each location were compared with each other location (Table 4 and Fig. 2). All comparisons show that the pairwise comparison of allele distribution are highly significant except for the comparison of Norton Sound, Alaska to Point Lay, Alaska and the Mackenzie Delta. This result may be due to a variety of reasons, most obviously that there may be no genetic variabilty among whales sampled at these locations. However, the significant difference between Point Lay, Alaska samples and the Mackenzie Delta samples suggests that the Alaska and Mackenzie Delta beluga are not part of a single, interbreeding population. We suspect that the failure to distinguish the Norton Sound beluga from the Point Lay and Mackenzie Delta beluga is due to the small number (n = 13) of Norton Sound whale samples tested.

CONCLUSIONS

This study has shown that the analysis of only one microsatellite locus has considerable population genetic resolving power. The data collected for four of the five sampling locations were in agreement with the Castle-Hardy-Weinberg equilibrium, indicating that null alleles were not generated with the amplification primers used. The St. Lawrence River beluga require examination of further microsatellite loci and more samples to evaluate the true level of genetic variability.

The number of alleles produced was sufficient to distinguish beluga sampled from western Arctic and eastern Arctic/subarctic locations. In addition, the method was able to reveal significant differences among beluga samples from Point Lay, Alaska, Mackenzie Delta, Nastapoka River, and St. Lawrence River.

The method was not able to distinguish beluga samples from Norton Sound, Alaska from the other two western Arctic locations examined (Pt. Lay, Alaska and Mackenzie Delta). However, the resolving power may be increased by the analysis of multiple microsatellite loci (Roy et al. 1994) which may reveal insight into the genetic variability among beluga sampled from more closely related populations.

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Table 1. Numbers of each allele found in beluga whales from Norton Sound Alaska, Point Lay Alaska, Mackenzie Delta, Nastapoka River, and the St. Lawrence River. (nt = nucleotides)

Location	Alleles				
	130 nt	132 nt	134 nt	136 nt	138 nt
Norton Sound, Alaska	2	0	21	3	0
Point Lay, Alaska	5	2	109	19	1
Mackenzie Delta	25	2	162	24	3
Nastapoka River	20	2	27	5	0
St. Lawrence River	33	0	1	0	0

Table 2. Probabilities of observing worse agreement by chance between observed frequencies of microsatellite genotypes and frequencies expected according to the Castle-Hardy-Weinberg equilibrium. A "-" indicates that only one allele was present, or that two alleles were detected but one was represented by only one copy.

Location	Probability test	Heterozygote deficit test
	p-value	*p*-value
Norton Sound, Alaska	1.0000	1.0000
Point Lay, Alaska	0.6665	1.0000
Mackenzie Delta	0.4362	1.0000
Nastapoka River	0.6299	0.4275
St. Lawrence River	-	-

Table 3. Distribution of alleles among male and female beluga and probabilities of a more divergent distribution.

	MACKENZIE DELTA		PT. LAY, ALASKA		NASTAPOKA RIVER	
Allele (nt=nucleotides)	M	F	M	F	M	F
130 nt allele	15	7	1	0	12	9
132 nt allele	0	3	1	0	2	1
134 nt allele	97	85	44	22	57	14
136 nt allele	20	9	6	6	10	1
138 nt allele	2	0	0	0	0	0
TOTAL NUMBER OF ALLELES	134	104	52	28	81	25
EXACT *p*- VALUE	0.0486		0.6011		0.0882	

Table 4. Exact probabilities calculated for contingency table pairwise comparisons of numbers of alleles found in samples of beluga from various geographic locations using pilot whale 464/465 microsatellite primers. Sample sizes are: Norton Sound n=13; Point Lay, Alaska n=50; Mackenzie Delta n=108; Nastapoka River n=26; St. Lawrence River n=17. (* indicates a western Arctic location, ** indicates an eastern Arctic/subarctic location)

	Point Lay (Alaska)*	Mackenzie Delta*	Nastapoka River**	St. Lawrence River**
Norton Sound (Alaska)*	0.655	0.964	0.012	0.0024
Point Lay (Alaska)	——	0.030	<0.0001	<0.0001
Mackenzie Delta	——	——	0.0001	<0.0001
Nastapoka River	——	——	——	<0.0001

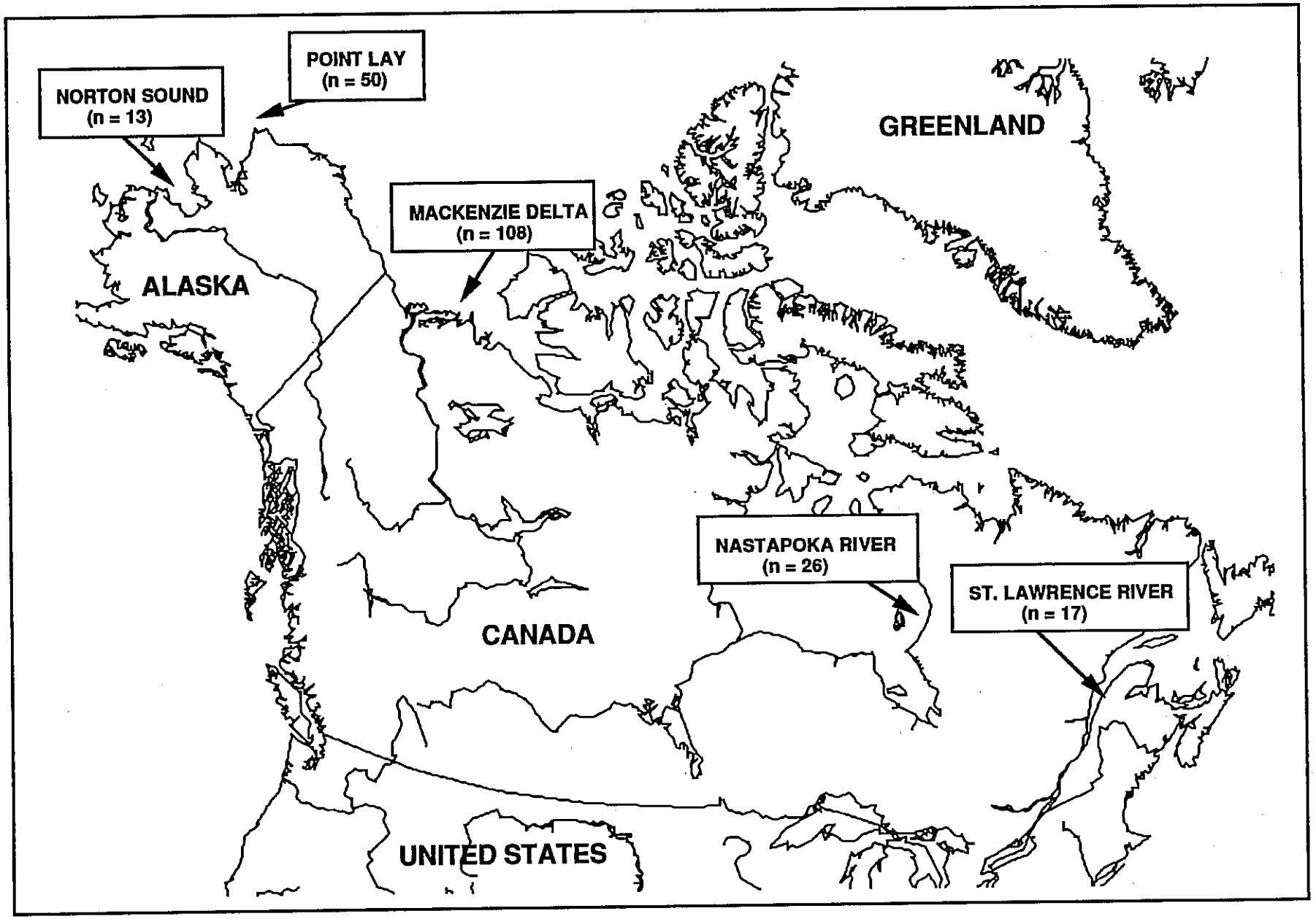

Fig. 1. Sample collection locations for beluga (*Delphinapterus leucas*) samples used in this study.

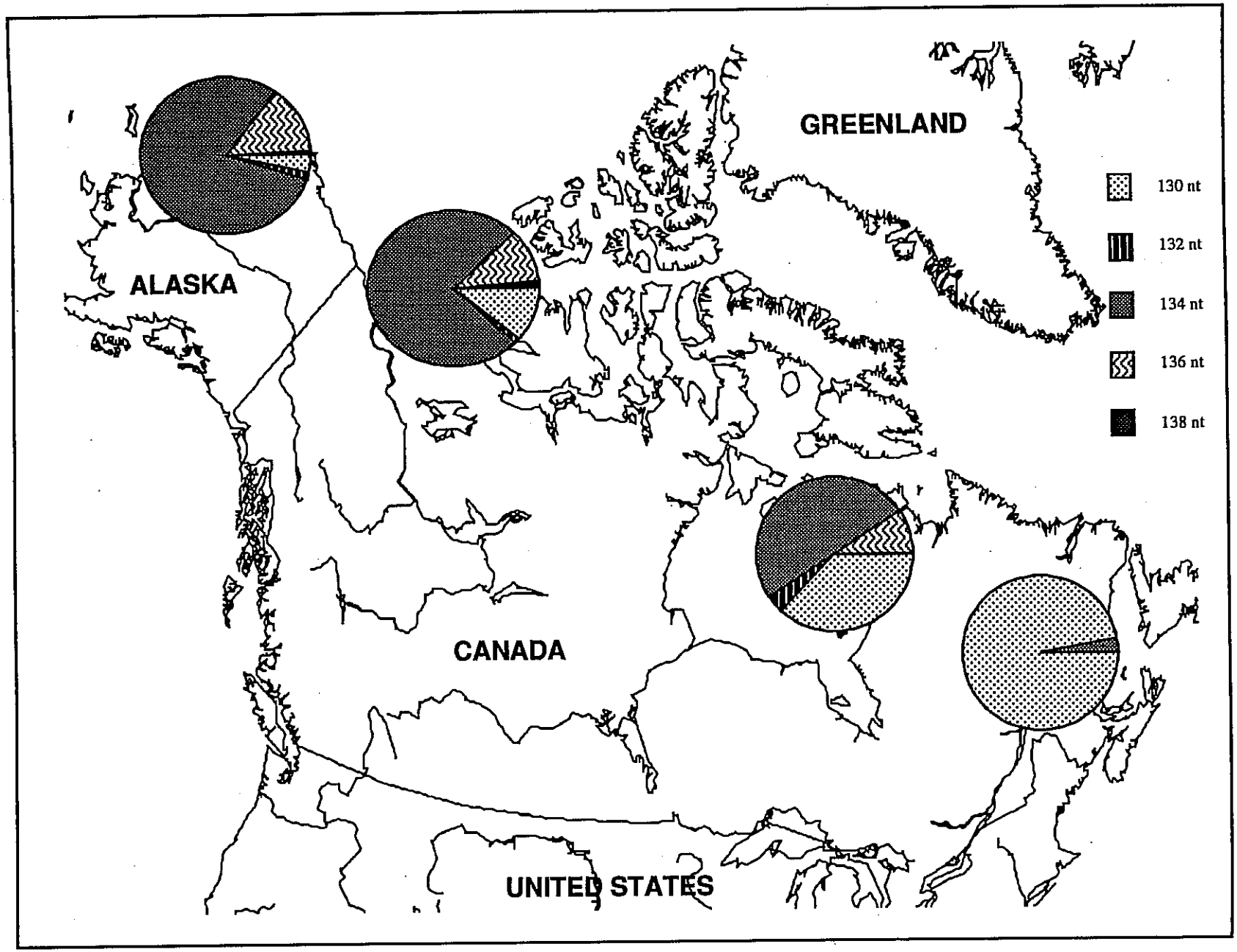

Fig. 2. Distribution of beluga whale (*Delphinapterus leucas*) microsatellite alleles among four of the geographic locations tested.