Molecular Ecology (2007) **16**, 1639–1648 doi: 10.1111/j.1365-294X.2007.03229.x

Panmictic population structure in the hooded seal (*Cystophora cristata*)

D. W. COLTMAN,* G. STENSON,† M. O. HAMMILL,‡ T. HAUG,§ C. S. DAVIS* and T. L. FULTON*
**Department of Biological Sciences, University of Alberta, Edmonton AB, Canada T6G 2E9, †Department of Fisheries and Oceans, PO Box 5667, St. John's NF, Canada A1C 3X9, ‡Department of Fisheries and Oceans, PO Box 1000, Mont Joli QC, Canada G5H 3Z4, §Institute of Marine Research, PO Box 6404, N-9294 Tromsø, Norway*

Abstract

Two putative populations of hooded seals (*Cystophora cristata*) occur in the North Atlantic. The Greenland Sea population pup and breed on the pack ice near Jan Mayen ('West Ice') while the Northwest Atlantic population is thought to pup in the Davis Strait, in the Gulf of St. Lawrence (the 'Gulf'), and off southern Labrador or northeast Newfoundland (the 'Front'). We used microsatellite profiling of 300 individuals at 13 loci and mitochondrial DNA sequencing of the control region of 123 individuals to test for genetic differentiation between these four breeding herds. We found no significant genetic differences between breeding areas, nor evidence for cryptic nor higher level genetic structure in this species. The Greenland Sea breeding herd was genetically most distant from the Northwest Atlantic breeding areas; however, the differences were statistically nonsignificant. Our data therefore suggest that the world's hooded seals comprise a single panmictic genetic population.

Keywords: F_{ST}, microsatellite, mitochondrial DNA, phylogeography, pinniped, population genetics

Received 26 August 2006; revision accepted 6 November 2006

Introduction

The hooded seal, *Cystophora cristata*, is a pelagic, deep-diving pinniped distributed throughout much of the North Atlantic and adjacent Arctic Oceans. Hooded seals breed synchronously during mid- to late March on the pack ice in four areas (Fig. 1): The Greenland Sea near Jan Mayen (also referred to as the 'West Ice'), in the Davis Strait between Baffin Island and western Greenland, in the Gulf of St. Lawrence (referred to as the 'Gulf'), and off southern Labrador and/or northeastern Newfoundland (the 'Front'). These four breeding herds are considered to belong to two putative populations (Anonymous 2006). The Greenland Sea population that births (whelps) near Jan Mayen is thought to constitute the Northeast Atlantic population while hooded seals whelping and breeding in Davis Strait, the Gulf and at the Front are all thought to belong to the Northwest Atlantic population (Anonymous 2006). The total Northwest Atlantic was estimated at 592 000 in 2005, of which over 90% are thought to whelp on the Front. The Greenland Sea population likely numbers between 70 000 and 90 000, however, there is considerable uncertainty around these estimates due to a paucity of data and limited understanding of the relationships between pupping areas (Anonymous 2006).

In the Northeast Atlantic, seals from the Greenland Sea whelping area disperse after breeding, ranging from the east Greenland coast to the Faroe Islands in the southeast and Svalbard in the northeast (Folkow *et al*. 1996). In the Northwest Atlantic, animals may spend 4–6 weeks feeding on the slope edge in the Gulf or off the Front or they move immediately to feed along continental shelves to the southwest of Iceland, or off southern Greenland continental shelves (Stenson *et al*. 2006b). By late June or early July, hooded seals congregate in the Denmark Strait where animals from both the Northeast Atlantic, and Northwest Atlantic were assumed to moult (Rasmussen 1960). Moulting animals have also been reported to the northwest of Jan Mayen Island (Nansen 1890, cited in Folkow *et al*. 1996) and recent satellite telemetry data suggest that Northeast Atlantic hooded seals moult to the northwest of Jan Mayen Island, while Northwest Atlantic animals moult in the Denmark Strait area off the Southeast Greenland coast (Folkow *et al*. 1996; Stenson, Hammill and Rosing-Asvid, unpublished data). Limited telemetry data, largely from juvenile animals,

Correspondence: D. W. Coltman, Fax: +1780-437-9234; E-mail: dcoltman@ualberta.ca

indicate that following the moult Northeast Atlantic hooded seals remain based in ice-covered waters off east Greenland, with excursions towards the Faroe Islands, Norway, Iceland and the United Kingdom (Folkow *et al*. 1996). In contrast, the majority of Northwest Atlantic adults appear to migrate around Cape Farwell and along west Greenland to Baffin Bay before returning to the whelping areas, while juvenile hooded seals appear to remain off Greenland. (Stenson, Hammill and Rosing-Asvid, unpublished data).

The relationships between the various whelping areas are poorly understood. Sergeant (1974) searched for, and discovered, the Davis Strait whelping concentration stimulated by comments from sealers and a theory put forth by Rasmussen (1960) that some seals that whelp off Newfoundland may breed further north in some years. It is not known if interbreeding occurs among the whelping areas within the Northwest Atlantic population, but seals from all three areas are known to mix during the nonbreeding period. Returns of pups tagged at the Front and in Davis Strait from the northeast Greenland moulting area (Stenson *et al*. 2006a) indicate that there can also be overlap between the two putative populations during the moulting period and therefore there is the potential for significant intermixing between stocks.

The limited evidence available thus far suggests a lack of sufficient reproductive isolation to facilitate the development of genetic differentiation between stocks. Analysis of skull morphology suggested limited genetic differentiation between the Front and Jan Mayen animals (Wiig & Lie 1984). Molecular investigations of the genetic population structure of hooded seals have also been undertaken using allozymes and multilocus DNA fingerprinting. Sundt *et al*. (1994) compared allele frequencies between Jan Mayen and the Front at three informative allozyme loci and found no significant differences. Levels of bandsharing between stocks were also not distinguishable from bandsharing within stocks (Sundt *et al*. 1994). On the basis of these findings, the hypothesis that there is considerable intermixing of stocks could not be rejected. However, neither allozymes nor DNA fingerprinting are ideal methods for studying population structure and stock assessment. Furthermore, previous studies have also not examined samples from Davis Strait nor from the Gulf of St. Lawrence.

In this study, we tested for genetic differentiation between all four breeding herds using microsatellite DNA profiling and mitochondrial DNA (mtDNA) sequencing. Microsatellites are ideal molecular markers for assessing population genetic structure at this spatial and temporal scale (Jarne & Lagoda 1996). They can also be used to assess whether each breeding herd is actually composed of a single breeding population, or whether there is cryptic population structure at a lower and undetected hierarchical level within each sampling unit (Pritchard *et al*. 2000). However, autosomal microsatellites will reveal the extent to which breeding areas are separated by gene flow as mediated by either sex, and therefore may not reflect the extent to which there may be sex-biased population structure (Prugnolle & de Meeus 2002), which may be relevant from the stock management perspective. We therefore also sequenced the control region of the mtDNA molecule from a subset of our samples to assess whether levels of genetic differentiation estimated by the two methods were consistent.

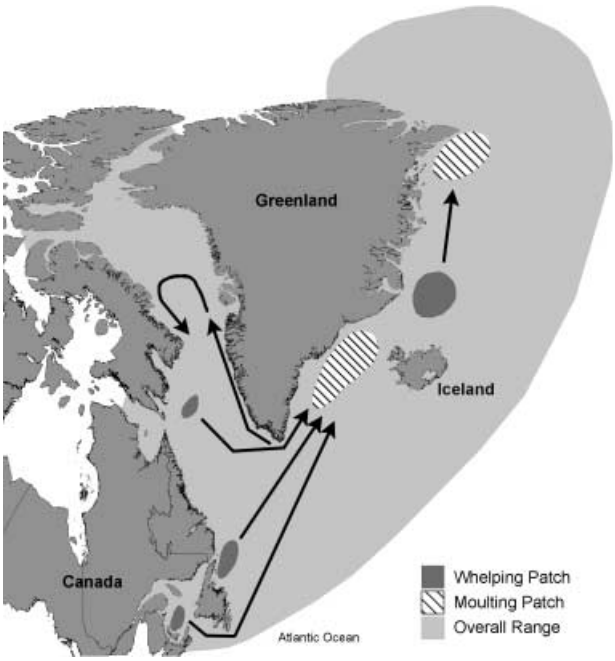

Fig. 1 Map of the North Atlantic indicating the locations of the whelping and moulting patches of the four subpopulations of the hooded seal.

Methods

Samples

Muscle and tissue samples were collected during tagging and sampling programs in the four main concentrations of breeding hooded seals in the North Atlantic (Fig. 1). For convenience, we henceforth refer to these four breeding areas as 'subpopulations'. To assure independence we only include genetic data from nonrelatives to the best of our knowledge (i.e. where a mother–pup pair were sampled on the ice we only include data from the pup). We present data from 300 individuals which comprise 108 samples collected from Davis Strait in 1984 ($N = 79$ pups and 29 adult males), 100 samples collected from the Front between 1990 and 2004 ($N = 31$ pups and 3 males from 1990; 9 adult females and 3 adult males from 1991; 8 adults males and 1 pup from 1992; 20 pups, 7 adult females and 4 adult males from 1994; 1 adult female from 2000; and 5 adult females and 3 adult males from 2004), 32 samples

Table 1 Microsatellite loci and multiplex PCR conditions. Twenty-one loci were amplified in eight reactions (1–8). Four reactions were loaded in each capillary injection (A or B)

Locus	Dye label	Primer concentration (nM)	PCR and capillary injection
Hg0*	VIC	150	excluded
Hg3.6*	6-FAM	100	excluded
Hg4.2*	6-FAM	100	A1
Hg6.1*	6-FAM	150	B5
Hg6.3*	PET	50	B8
Hg8.9*	6-FAM	100	A1
Hg8.10*	NED	50	B7
Hgdii*	PET	50	B8
Hl8†	VIC	100	A2
Hl16†	VIC	100	A2
Hl20†	NED	200	A3
Lc26†	6-FAM	150	B5
Lc28†	NED	50	B7
Lw7†	PET	50	A4
Lw10†	NED	50	B7
Pvc19‡	6-FAM	150	B5
Pvc26‡	VIC	150	B6
Pvc30‡	VIC	100	A2
Pvc63‡	PET	50	A4
Pvc78‡	NED	200	A3
Pv9§	PET	50	B8
Pv10§	VIC	150	B6
Pv11§	NED	200	A3
Pv16§	6-FAM	100	excluded

*Allen *et al*. 1995; †Davis *et al*. 2002; ‡Coltman *et al*. 1996; §Goodman 1997.

from the Gulf of St. Lawrence in 2005 (16 adults females and 16 adult males), and 60 samples from the Greenland Sea in 2002 (all pups). DNA was extracted using QIAamp spin column tissue extraction kits following the manufacturer's instructions (QIAGEN).

Microsatellite genotyping

We selected 24 microsatellite loci developed in phocid seals for genotyping hooded seals (Table 1). The expected allele size range for each locus and the use of the Applied Biosystems 5-dye (DS-33) fragment analysis system allowed for these 24 loci to be loaded in two capillary injections. During initial testing three loci failed to amplify and were excluded from additional analysis (Hg0, Hg3.6 and Pv16). The remaining 21 loci were amplified in eight multiplexed reactions. Polymerase chain reactions (PCR) contained approximately 75 ng of genomic template DNA, 1× QIAGEN multiplex PCR master mix (QIAGEN) and 50–200 nM (each primer) in a total reaction volume of 10 μL. Reactions were performed in Eppendorf Mastercycler thermocyclers under the following conditions; 95 °C for 15 min followed by 33 cycles of 94 °C for 30 s, 57 °C for 90 s and 72 °C for 90 s followed by a final extension at 72 °C for 10 min. Resulting products were resolved in POP-4 polymer on a 36-cm capillary array using a 3100-Avant Genetic Analyser and sized using GS500 LIZ internal size standard and GENEMAPPER software (Applied Biosystems). Three loci (Pvc 19, Pvc 30 and Pvc 63) failed to amplify in a large proportion (> 20%) of the individuals tested and were excluded from further analysis.

Microsatellite data analysis

We first assessed levels of variation at each locus and estimated F_{IS} (Weir & Cockerham 1984) for the entire sample using FSTAT 2.93 (Goudet 1995). The statistical significance of F_{IS} was assessed using randomization tests in FSTAT (Goudet 1995). The frequency of null alleles was estimated for each locus from the global heterozygote excess using the algorithm described in (Summers & Amos 1997) and implemented by CERVUS (Marshall *et al*. 1998). Loci that showed high and outlying null allele frequency estimates and F_{IS} were excluded from further analyses. We tested for linkage disequilibrium between all pairs of loci over all areas using exact tests implemented by GENEPOP (Raymond & Rousset 1995).

We quantified genetic variability within each subpopulation by allelic richness (k), observed and expected heterozygosity (H_O and H_E) calculated by FSTAT (Goudet 1995). Homogeneity of genetic variation among subpopulations was tested using Wilcoxon signed rank tests. Departures from Hardy–Weinberg equilibrium were examined using exact tests (Guo & Thompson 1992) using a Markov chain as implemented by GENEPOP (Raymond *et al.* 1995). Loci were combined using Fisher's method to examine departure from equilibrium for each area. Exact tests were performed for allele frequency differences between all pairs of subpopulations and over all subpopulations using GENEPOP (Raymond *et al*. 1995). Genetic divergences between subpopulations were quantified using F_{ST} implemented by FSTAT (Weir *et al.* 1984; Goudet 1995). In all cases we interpreted statistical significance after correcting for multiple comparisons by the Bonferroni method (Zar 1996).

We used the Bayesian methodology of STRUCTURE (Pritchard *et al*. 2000) to determine the level of genetic substructure in the dataset independently of sampling areas. We assumed an admixture model with correlated allele frequencies (Falush *et al*. 2003). To estimate the number of subpopulations (K), 10 independent runs of K = 1–12 were carried out at 1 000 000 Markov chain Monte Carlo (MCMC) repetitions following a burn-in of 500 000 repetitions. The most probable number of subpopulations was taken using the highest mean log-likelihood of K.

Mitochondrial DNA control region sequencing

Approximately 60 individuals from each of the two putative stocks were randomly selected for mitochondrial control region sequencing. All of the Greenland Sea individuals were included ($n = 60$), plus 21 individuals from each of the remaining three subpopulations. An approximately 900-bp fragment encompassing the end of cytochrome *b*, $tRNA_{thr}$, $tRNA_{pro}$, and part of hypervariable region I (HVR I) in the control region was amplified following (Delisle & Strobeck 2005), using their primers mtDloopU (5′-CTAACATGAATCGGAGGACAACCAG-3′) and mt10bL (5′-ATTTGACTGCGTCGAGACCTTTA-3′) with an annealing temperature of 52 °C. Amplified products were purified with the QIAquick PCR purification kit (QIAGEN) and directly sequenced using BigDye version 3.1.1 (Applied Biosystems) for both the amplification primer mtDloopU and a new reverse sequencing primer (5′-CCTGAAGTAAGAACCAGATG-3′) approximately 650 bp downstream from the upper primer. Dye terminator removal was performed using QIAGEN DyeEx 96 kit and fragments were resolved using an Applied Biosystems 3730 capillary sequencer.

Mitochondrial DNA control region sequence analysis

Sequences were analysed and basecalled with FOUNDATION DATA COLLECTION version 3.0 and aligned by eye in SEQUENCE NAVIGATOR version 1.0.1 (both from Applied Biosystems). The number of polymorphic sites, nucleotide diversity π (Nei 1987), number of haplotypes, and haplotypic diversity (*h*) were determined for each subpopulation and the total population using ARLEQUIN 3.01 (Excoffier *et al*. 2005). Analysis of molecular variance, AMOVA (Excoffier *et al*. 1992) was used to examine how variation is partitioned within and among subpopulations and to determine population differentiation for all pairwise comparisons. AMOVA is an analogue of Wright's hierarchical *F*-statistics, calculating Φ-statistics that incorporate evolutionary distance between haplotypes in addition to frequency data. Significance of the AMOVA was determined using a null distribution obtained from 1023 nonparametric permutations for the total population and 110 permutations for the pairwise comparisons (Excoffier *et al*. 1992). Exact tests for population differentiation based on sample haplotype frequencies were performed in ARLEQUIN 3.01 for all pairwise population comparisons.

A neighbour-joining (NJ) tree (Saitou & Nei 1987) using the Tamura & Nei (1993) DNA evolution model for control region sequence was constructed in PAUP* 4.0b10 (Swofford 2003). Nodal support was estimated by 1000 bootstrap replicates. Relationships between haplotypes were analysed with a statistical parsimony network constructed using TCS 1.21 (Clement *et al*. 2000) and a minimum spanning network based on the squared distance (Tamura-Nei) matrix used in Φ_{ST} calculations in ARLEQUIN 3.01 (Excoffier 2005). To test for population expansion, Fu's F_{ST} and the raggedness index were calculated in DNASP (Rozas *et al*. 2003), with significance values for both estimated using distributions from 1000 replicate coalescent simulations.

Results

Microsatellites

We observed high levels of genetic variation over all subpopulations pooled (Table 2). The number of alleles per locus in the total sample ranged from 5 to 15 (mean = 12.0),

Locus	Alleles	*n*	Allele size range	H_O	H_E	F_{IT}	*P*	Null allele frequency
Hg4.2	15	299	146–172	0.77	0.76	−0.01	0.62	−0.009
Hg6.1	10	300	135–161	0.41	0.40	−0.02	0.75	−0.010
Hg6.3	15	300	214–242	0.85	0.86	0.02	0.19	0.010
Hg8.1	18	299	162–196	0.91	0.90	−0.02	0.81	−0.008
Hg8.9	15	293	180–210	0.88	0.90	0.02	0.17	0.011
HgDII	15	300	117–149	0.82	0.83	0.01	0.38	0.004
Hl16	8	299	129–143	0.69	0.65	−0.06	0.97	−0.040
Hl20	13	292	98–122	0.78	0.81	0.03	0.13	0.016
Hl8	13	300	98–120	0.80	0.83	0.04	0.06	0.023
Lc26	**14**	**293**	**290–328**	**0.77**	**0.85**	**0.10**	**<0.005**	**0.050**
Lc28	**12**	**291**	**116–146**	**0.71**	**0.82**	**0.14**	**<0.005**	**0.069**
Lw10	**8**	**290**	**101–117**	**0.43**	**0.70**	**0.38**	**<0.005**	**0.238**
Lw7	14	298	150–178	0.71	0.74	0.04	0.08	0.026
Pv10	**5**	**286**	**119–129**	**0.29**	**0.51**	**0.44**	**<0.005**	**0.286**
Pv9	8	300	160–180	0.60	0.64	0.06	0.04	0.034
Pvc26	8	300	100–116	0.43	0.44	0.02	0.37	0.015
Pvc78	13	299	122–154	0.73	0.76	0.05	0.04	0.024

Table 2 Polymorphism characteristics of 17 microsatellite loci amplified in western Atlantic hooded seals pooled from four subpopulations. The *P* value refers to the proportion of randomizations that gave a larger F_{IT} than observed. Loci indicated in bold show significant heterozygote excess following Bonferroni correction for multiple comparisons

Table 3 Mean polymorphism characteristics for 13 loci in four subpopulations of hooded seals from the western Atlantic. Allelic richness (*k*) is based on a rarefaction analysis of 31 individuals per population conducted in FSTAT (Goudet 1995)

Subpopulation	*n*	*k*	H_O	H_E	F_{IS}
Davis Strait	108	8.99	0.72	0.73	0.015
The Front	100	9.17	0.71	0.73	0.029
Gulf of St. Lawrence	32	9.10	0.75	0.73	−0.021
Greenland Sea	60	9.16	0.73	0.74	0.017

Table 4 *F*-statistics describing the population structure of western Atlantic hooded seals divided into four subpopulations. *$P < 0.05$

Locus	F_{IS}	F_{ST}
HG4.2a	−0.009	−0.0015
HG6.1a	−0.023	−0.0029
HG6.3a	0.017	0.0036*
HG8.1a	−0.018	0.0032
HG8.9a	0.025	−0.0008
HGDIIa	0.010	−0.0010
HL16a	−0.067	0.0044
HL20a	0.035	−0.0026
HL8a	0.043	−0.0012
LW7a	0.040	−0.0027
PV9a	0.064	−0.0036
PVC26a	0.019	0.0013
PVC78a	0.050	−0.0014
All	0.016	−0.0003

observed heterozygosity ranged from 0.29 to 0.91 (mean = 0.68) and expected heterozygosity ranged from 0.40 to 0.90 (mean = 0.73). Significant excess homozygosity was observed at four loci (Lc26, Lc28, Lw10, Pv10) in the pooled sample ($F_{IT} \geq 0.10$) and in each subpopulation which suggested that null or nonamplifying alleles were present at reasonably high frequency (≥ 0.05). We therefore removed these four loci from subsequent analyses of population structure. Analyses including these loci gave identical results to those shown below. We did not observe significant linkage disequilibrium between any pair of loci after correction for multiple comparisons.

We observed similar levels of genetic variation within each subpopulation at the 13 loci used to characterize population structure (Table 3). Allelic richness, observed and expected heterozygosity were statistically indistinguishable between subpopulations ($P > 0.8$ for each). Neither F_{IS} nor F_{ST} differed significantly from zero at each locus after correcting for multiple comparisons (Table 4). Combined over 13 loci F_{IS} was +0.016 ($P > 0.05$) and F_{ST} was –0.0003 ($P > 0.05$). Allele frequencies did not differ significantly between any pair of subpopulations (combined $P = 0.13$; Table 5) nor did they differ significantly over all four subpopulations (combined $P = 0.37$). Pairwise F_{ST} ranged from –0.0022 to 0.0009 (Table 5). The most likely number of subpopulations identified by the STRUCTURE analysis was $K = 1$ (data not shown) and higher values of *K* always returned lower likelihoods. At higher *K* mean levels of individual admixture (*q*) were also low and declined monotonically (data not shown).

Table 5 Pairwise differentiation (F_{ST} above diagonal) and combined probability test for allele frequency differences (*P* value below diagonal) between subpopulations

	Davis Strait	The Front	Gulf of St. Lawrence	Greenland Sea
Davis Strait		−0.0005	−0.0022	0.0003
The Front	0.59		−0.0003	0.0001
Gulf of St. Lawrence	0.54	0.45		0.0009
Greenland Sea	0.36	0.13	0.54	

Mitochondrial DNA control region

A final alignment of 541 bp was obtained, containing 66 bp of the 3′ end of cytochrome *b*, complete $tRNA_{thr}$ and $tRNA_{pro}$, and 339 bp (aligned) into the HVR I of the control region. A region starting 8 bp into HVR I was excluded from the final alignment due uncertain alignment of a poly C repeat ranging from 2 to approximately 14 bp in length. In many individuals, this repeat was too long to be directly sequenced, thus, many sequences are single stranded with the upper and lower sequences terminating within this repeat region. Two single base insertion-deletion events (indels) were observed in Greenland Sea individual U20020504, one deletion in $tRNA_{pro}$ and one insertion in HVR I. Indels were otherwise only observed in a 7-bp region immediately following the aforementioned repeat region at the beginning of the control region.

All subpopulations showed extremely high numbers of haplotypes relative to the number of individuals observed (Table 6). Of 123 individuals, only 12 haplotypes were observed more than once. Five instances were observed within populations (two in the Front, three in Greenland), five were observed between populations, and two haplotypes were observed more than twice. One of these haplotypes was observed six times (four in Greenland, one in each the Front and Davis Straight) and the other was observed four times (twice in Greenland, once each in the Gulf and Davis Straight). Each population shared at least one haplotype with every other population. Consequently, the haplotype diversity approached or equalled 1.0 for both the total population and for all individual subpopulations (Table 6). In contrast, nucleotide diversity was relatively low and similar across subpopulations, as the subpopulation estimates

Table 6 Number of individuals, number of haplotypes, haplotype diversity (h), number of polymorphic sites, and nucleotide diversity (π) for each subpopulation and the total population

Subpopulation	Number of individuals	Number of haplotypes	$h \pm$ SE	Number of polymorphic sites	$\pi \pm$ SE
Davis Strait	21	21	1.000 ± 0.015	53	0.022 ± 0.012
The Front	21	19	0.991 ± 0.018	48	0.021 ± 0.011
Gulf of St. Lawrence	21	21	1.000 ± 0.015	54	0.021 ± 0.011
Greenland Sea	60	53	1.000 ± 0.003	77	0.019 ± 0.010
All	123	105	1.000 ± 0.001	91	0.023 ± 0.001

Table 7 Genetic differentiation between populations as measured by pairwise Φ_{ST} (above diagonal) and exact test for differentiation based on haplotype frequencies (P values below diagonal)

	Davis Strait	The Front	Gulf of St. Lawrence	Greenland Sea
Davis Strait		−0.0241	−0.0182	0.0076
The Front	0.23		−0.0084	0.0061
Gulf of St. Lawrence	1.00	0.24		0.0022
Greenland Sea	1.00	0.39	1.00	

ranged from 0.019 ± 0.010 (Greenland Sea) to 0.022 ± 0.012 (Gulf of St. Lawrence) and the total population was 0.023 ± 0.011. Number of polymorphic sites ranged from 8.9% of the 541 aligned bases (Front, $n = 21$) to 14.2% (Greenland Sea, $n = 60$). Across all subpopulations, 91 (16.8%) polymorphic sites were observed (Table 6).

No significant differentiation was observed between subpopulations. AMOVA indicated that 100.07% of the variation in the total population was found within subpopulations, resulting in –0.07% of the variation between subpopulations and a global Φ_{ST} of –0.00068 ($P = 0.49$). Pairwise subpopulation AMOVA calculations also showed no significant differences at $\alpha = 0.05$. Pairwise comparisons of the Greenland Sea subpopulation to other subpopulations yielded slightly higher Φ_{ST} values than the other pairwise comparisons but were not significant ($P > 0.20$). All comparisons between the three remaining subpopulations yielded negative Φ_{ST} values close to zero (Table 7), with P values greater than 0.5. Exact tests also showed no significant differentiation between any pair of populations at $\alpha = 0.05$ (Table 7). When the subpopulations were grouped to represent the Greenland Sea population and the combined Northwest Atlantic population, the results of all tests were nearly identical to analyses performed using four subpopulations. AMOVA partitioned 99.06% of the variation within populations and the global Φ_{ST} of 0.00938 was nonsignificant ($P = 0.06$). For the population as a whole, both the raggedness index ($r = 0.0034$) and Fu's F_{ST} ($F_{ST} = -127.048$) were significant with $P \leq 0.001$.

Visualization of evolutionary relationships between individuals did not indicate any structure within the total population. The NJ tree (Fig. 2) and minimum spanning network (result not shown) were both fairly starlike, with few relationships supported by bootstrapping in the NJ tree and no clear clustering of haplotypes from any single subpopulation. The parsimony network was equally unstructured with regard to subpopulation and was highly reticulated (result not shown).

Discussion

Our microsatellite and mtDNA analyses indicate considerable genetic variation in the hooded seal. However, all of the variation was partitioned within, rather than among, subpopulations for both microsatellites and mtDNA and the four hooded seal breeding groups show minimal and statistically nonsignificant genetic differentiation at both nuclear (microsatellite) and mitochondrial genetic markers. Bayesian analysis using the STRUCTURE program also failed to reveal cryptic population substructure. Our results therefore confirm previous findings that suggested a lack of genetic differentiation and considerable intermixing between Newfoundland and Jan Mayen (Sundt *et al*. 1994) and extend this conclusion to Davis Strait and the Gulf of St. Lawrence.

The high haplotype diversity ($h \approx 1.0$) relative to nucleotide diversity (2.3%) we observed in hooded seals is not unusual compared to other historically abundant pinniped species. For example, Pacific Harbor seals were observed to have high haplotypic diversity ($h = 0.975$) and moderate nucleotide diversity ($\pi = 1.47\%$) by Westlake & O'Corry-Crowe (2002). Other otariid species such as *Eumetopias jubatus* ($h = 0.9164$; Baker *et al*. 2005), *Arctocephalus pusillus* ($h = 0.975$; Matthee *et al*. 2006), and even prebottleneck *Arctocephalus townsendi* ($h = 0.997$; Weber *et al*. 2004) show similar trends.

High levels of genetic diversity and little genetic structure are not unexpected for pinniped species that breed on pack ice. This may be due the unpredictable and unstable nature of the breeding habitat which does not facilitate natal fidelity nor highly polygynous mating systems, resulting

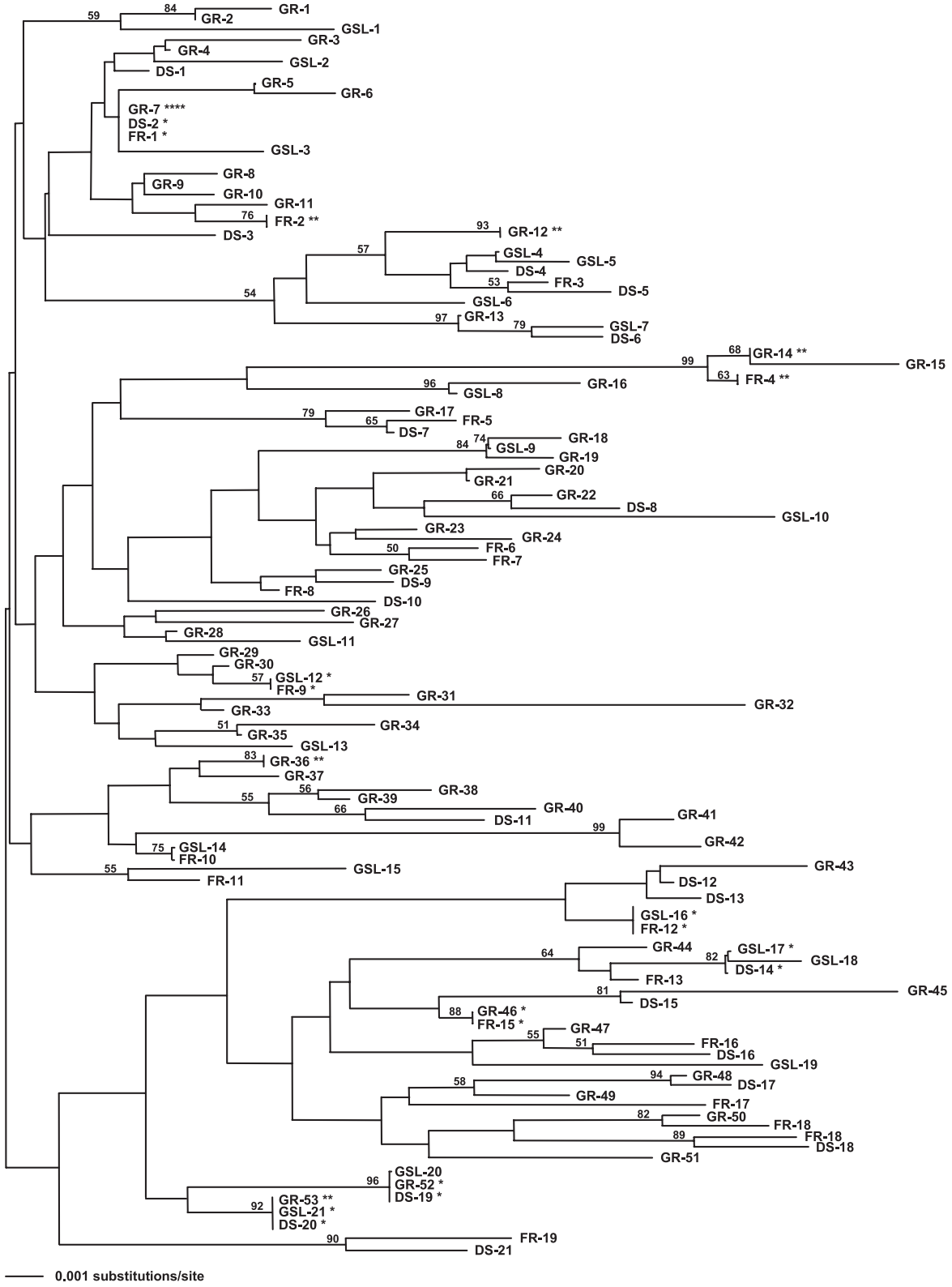

Fig. 2 Hidpoint-rooted neighbour-joining tree of individual mitochondrial control region haplotypes using Tamura–Nei distance, with associated nodal bootstrap support. The notation (*) indicates a taxon that shares an identical haplotype with another individual. The number of stars indicates the number of times that haplotype was observed within a population. DS, Davis Strait; GR, Greenland Sea; GSL, Gulf of St. Lawrence; FR, Front.

in a panmictic and large breeding population (Stirling 1975; Stirling 1983; Davis 2004). Pack-ice breeding Antarctic species including the Ross seal (*Ommatophoca rossii*), leopard seal (*Hydrurga leptonyx*) and crabeater seal (*Lobodon carcinophagus*) also show little genetic structure ($F_{ST} \approx 0$; Davis 2004). Conversely, land-breeding pinnipeds show significant population structure consistent with natal fidelity to predictable breeding habitat [e.g. harbor seals, *Phoca vitulina* (Stanley *et al*. 1996; Westlake & O'Corry-Crowe 2002); southern elephant seals, *Mirounga leonina* (Hoelzel *et al*. 2001)]. Grey seals (*Halichoerus grypus*) breed on both land and ice. Land-breeding grey seals show evidence of genetic structure between colonies in Britain (Allen *et al*. 1995), and between the western North Atlantic, Baltic and Norwegian populations (Boskovic *et al*. 1996). However, in the western North Atlantic the pack-ice breeding population of the Gulf of St. Lawrence is genetically indistinguishable from the land-breeding population of Sable Island (Boskovic *et al*. 1996).

The harp seal (*Pagophilus groenlandica*) is a pack-ice breeding phocid that has a similar geographical distribution and life-history to the hooded seal. However, Northeast and Northwest stocks of harp seals have been clearly identified using allozymes, multilocus DNA fingerprinting and mtDNA (Meisfjord & Sundt 1996; Perry *et al*. 2000). These studies indicate that while harp seals of the Northwest Atlantic sampled from the Gulf of St. Lawrence and the Front were genetically indistinguishable, as were harp seals from the Greenland Sea and White Sea of the Northeast Atlantic, the genetic differentiation between the Northeast and Northwest populations was considerable (i.e. $F_{ST} = 0.12$ based on mtDNA reported in Perry *et al*. 2000 compared to $\Phi_{ST} \approx 0.01$ for hooded seals reported here). In hooded seals, the greatest degree of differentiation was observed between the Greenland Sea and the subpopulations of the Northwest Atlantic for both microsatellite and mtDNA (Table 4 and Table 6, respectively). However, these differences, as well as tests for differentiation between the Greenland Sea and the northwest populations pooled (for mtDNA $\Phi_{ST} = 0.0094$, $P = 0.06$; for microsatellites $F_{ST} = 0.006$, test for allele frequency differences $P = 0.21$), were also nonsignificant.

One possible explanation for this difference is that hooded seals may have relatively recently recolonized much of their range following the last glacial period and have either had insufficient time or sufficient gene flow and population mixing to prevent genetic differentiation. The mitochondrial data bears the hallmark signature of a recent founding followed by a period of rapid population growth. Features supporting this interpretation include the lack of geographical structuring and star genealogy apparent in the phylogenetic tree (Fig. 2) and the low nucleotide diversity (2.3%) relative to the high haplotype diversity (1.0). This pattern arises because relatively few lineages are lost in a rapidly expanding population (Avise 1994). Statistical tests for population expansion based on the distribution of pairwise nucleotide differences or mismatches further support this interpretation. Our data set indicated a very low raggedness statistic $r = 0.0034$ [deviating from the expected value for a stationary population generated by simulation at $P = 0.001$ (Rozas *et al*. 2003)], which indicates a unimodal mismatch distribution consistent with the signature of an expanding population (Rogers & Harpending 1992; Harpending 1994). Fu's F_{ST} was large and negative ($F_s = -127.048$; $P < 0.001$) which is also consistent with an expanding population (Fu 1997). A similar signature of recent expansion has also been observed in the mtDNA of the ice breeding Pacific harbor seal which also likely expanded and recolonized northwards following the retreat of the Pleistocene ice sheets (Westlake & O'Corry-Crowe 2002).

In summary, mtDNA and microsatellite analyses indicate that the world's population of hooded seals could be considered a panmictic breeding population. There is clearly sufficient gene flow to prevent genetic differentiation, and therefore the breeding herds are not genetically isolated. However, it is important to note that sufficient gene flow to prevent genetic differentiation does not necessarily equate to demographic and population dynamic coupling of the four breeding herds. Relatively few migrants per generation are sufficient to prevent genetic drift from causing the breeding herds from becoming genetically differentiated. Although there are very little data available, the well-known wandering behaviour of hooded seal juveniles would provide a mechanism for exchange (Hammill 1993; Mignucci-Giannoni & Odell 2001). At the same time, satellite telemetry and tagging data indicate a degree of fidelity to breeding herds (Hammill 1993; Folkow *et al*. 1996). It would therefore be prudent to continue to manage the stocks separately given the paucity of data on abundance, population dynamics and the demographic independence of hooded seal breeding herds.

Acknowledgements

Jennifer Bonneville carried out the microsatellite DNA analyses. F. Kapel, W. Penney, D. McKinnon, D. Wakeham and N-E. Skavberg assisted in obtaining the samples. This work was supported by NSERC (Discovery Grant to D.W.C.), DFO and Alberta Ingenuity (D.W.C.). We thank Bob Wayne and two anonymous referees for their constructive comments on our manuscript.

References

Allen PJ, Amos W, Pomeroy PP, Twiss SD (1995) Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. *Molecular Ecology*, **4**, 653–662.

Anonymous (2006) *Report of the ICES/NAFO Working Group on Harp and Hooded Seals (WGHARP)*, 28 pp. International Council for the Exploration of the Sea, Copenhagen.

Avise JC (1994) *Molecular Markers, Natural History and Evolution*. Chapman & Hall, London.

Baker AR, Loughlin TR, Burkanov V *et al.* (2005) Variation of mitochondrial control region sequences of Steller sea lions: the three-stock hypothesis. *Journal of Mammalogy*, **86**, 1075–1084.

Boskovic R, Kovacs KM, Hammill MO, White BN (1996) Geographic distribution of mitochondrial DNA haplotypes in grey seals (*Halichoerus grypus*). *Canadian Journal of Zoology-Revue Canadienne de Zoologie*, **74**, 1787–1796.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.

Coltman DW, Bowen WD, Wright JM (1996) PCR primers for harbour seal (*Phoca vitulina concolour*) microsatellites amplify polymorphic loci in other pinniped species. *Molecular Ecology*, **5**, 161–163.

Davis CS (2004) *Phylogenetic Relationships of the Phocidae and Population Genetics of Ice Breeding Seals*. PhD, University of Alberta, Edmonton, Canada.

Davis CS, Gelatt TS, Siniff D, Strobeck C (2002) Dinucleotide microsatellite markers from the Antarctic seals and their use in other Pinnipeds. *Molecular Ecology Notes*, **2**, 203–208.

Delisle I, Strobeck C (2005) A phylogeny of the Caniformia (order Carnivora) based on 12 complete protein-coding mitochondrial genes. *Molecular Phylogenetics and Evolution*, **37**, 192–201.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes – application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Excoffier L, Laval G, Schneider S (2005) ARLEQUIN version 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Folkow LP, Martensson PE, Blix AS (1996) Annual distribution of hooded seals (*Cystophora cristata*) in the Greenland and Norwegian Seas. *Polar Biology*, **16**, 179–189.

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Goodman SJ (1997) Dinucleotide repeat polymorphisms at seven anonymous microsatellite loci cloned from the European harbour seal (*Phoca vitulina vitulina*). *Animal Genetics*, **28**, 310–311.

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics*, **48**, 361–372.

Hammill MO (1993) Seasonal movements of hooded seals tagged in the Gulf of St. Lawrence, Canada. *Polar Biology*, **13**, 307–310.

Harpending HC (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology*, **66**, 591–600.

Hoelzel AR, Campagna C, Arnbom T (2001) Genetic and morphometric differentiation between island and mainland southern elephant seal populations. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **268**, 325–332.

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology & Evolution*, **11**, 424–429.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Matthee CA, Fourie F, Oosthuizen WH, Meyer MA, Tolley KA (2006) Mitochondrial DNA sequence data of the Cape fur seal (*Arctocephalus pusillus pusillus*) suggest that population numbers may be affected by climatic shifts. *Marine Biology*, **148**, 899–905.

Meisfjord J, Sundt RC (1996) Genetic variation between populations of the harp seal, *Phoca groenlandica*. *Ices Journal of Marine Science*, **53**, 89–95.

Mignucci-Giannoni AA, Odell DK (2001) Tropical and subtropical records of hooded seals (*Cystophora cristata*) dispel the myth of extant Caribbean monk seals (*Monachus tropicalis*). *Bulletin of Marine Science*, **68**, 47–58.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Perry EA, Stenson GB, Bartlett SE, Davidson WS, Carr SM (2000) DNA sequence analysis identifies genetically distinguishable populations of harp seals (*Pagophilus groenlandicus*) in the northwest and northeast Atlantic. *Marine Biology*, **137**, 53–58.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Prugnolle F, de Meeus T (2002) Inferring sex-biased dispersal from population genetic tools: a review. *Heredity*, **88**, 161–165.

Rasmussen B (1960) On the stock of hood seals in the northern Atlantic. *Fish. Res. Board of Canada Translation Series No. 387*.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

Rozas J, Sanchez-DeI, Barrio JC, Messeguer X, Rozas R (2003) DNASP: DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.

Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Sergeant DE (1974) A rediscovered whelping population of hooded seals, *Cystophora cristata* Erxleben and its possible relationships to other populations. *Polarforschung*, **44**, 1–7.

Stanley HF, Casey S, Carnahan JM *et al.* (1996) Worldwide patterns of mitochondrial DNA differentiation in the harbor seal (*Phoca vitulina*). *Molecular Biology and Evolution*, **13**, 368–382.

Stenson GB, Sjare BS, Potelov V (2006a) Tagging of the northwest Atlantic hooded seals (1983–2006): implication for stock structure. In: *Meeting of the Joint ICES/NAFO Working Group on Harp and Hooded Seals, 2006*. pp. SEA–157. Copenhagen, Denmark.

Stenson GB, Hammill MO, Poyot F, Fedak M (2006b) The seasonal distribution of hooded seals, *Cystophora cristata*, in the northwest Atlantic. In: *Meeting of the Joint ICES/NAFO Working Group on Harp and Hooded Seals*, pp. SEA–159. Copenhagen, Denmark.

Stirling I (1975) Factors affecting the evolution of social behaviour in the Pinnipedia. *Rapp. P.-V. Reun. Conservation. Int. Explor. Mer.*, **169**, 205–212.

Stirling I (1983) The evolution of mating systems in pinnipeds. In: *Advances in the Study of Mammalian Behaviour* (eds Eisenberg JF, Kleiman DG), pp. 489–527. American Society of Mammalogists, Shippensburg, Pennsylvania.

Summers K, Amos W (1997) Behavioral, ecological, and molecular genetic analyses of reproductive strategies in the Amazonian dart-poison frog, Dendrobates ventrimaculatus. *Behavioral Ecology*, **8**, 260–267.

Sundt RC, Dahle G, Naevdal G (1994) Genetic variation in the hooded seal, *Cystophora cristata*, based on enzyme polymorphism and multilocus DNA fingerprinting. *Hereditas*, **121**, 147–155.

Swofford DL (2003) PAUP*. *Phylogenetic Analysis Using Parsimony (*and Other Methods) version 4.0b10*. Sinauer Associates, Sunderland, Massachusetts.

Tamura L, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

Weber DS, Stewart BS, Lehman N (2004) Genetic consequences of a severe population bottleneck in the Guadalupe fur seal (*Arctocephalus townsendi*). *Journal of Heredity*, **95**, 144–153.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Westlake RL, O'Corry-Crowe GM (2002) Macrogeographic structure and patterns of genetic diversity in harbor seals (*Phoca vitulina*) from Alaska to Japan. *Journal of Mammalogy*, **83**, 1111–1126.

Wiig O, Lie RW (1984) An analysis of the morphological relationships between the hooded seals (*Cystophora cristata*) of Newfoundland, the Denmark Strait, and Jan Mayen. *Journal of Zoology*, **203**, 227–240.

Zar JH (1996) *Biostatistical Analysis*. 3rd edn. Prentice Hall, London.