Microsatellite assessment of walrus (*Odobenus rosmarus rosmarus*) stocks in Canada

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ABSTRACT

Walruses in Canada are currently subdivided into seven stocks based on summering areas; Western Jones Sound (WJS), Baffin Bay (BB), Penny Strait–Lancaster Sound (PS-LS), North Foxe Basin (N-FB), Central Foxe Basin (C-FB), Hudson Bay Davis Strait (HB-DS) and Southern and Eastern Hudson Bay (SE-HB). In this study, walrus were sampled from six of the seven stocks (SE-HB samples were not available) and genotyped at 10 microsatellite loci. All stocks were genetically diverse (average heterozygosity of 0.58) with no evidence of inbreeding (average F_{IS} of 0.03). We detected significant genetic differentiation among the stocks and a pattern of genetic spatial autocorrelation that suggests a moderate effect of geographic distance on gene flow among stocks. Bayesian clustering suggested the six recognized stocks were elements of two larger genetic clusters—a northern Arctic population (containing BB, WJS, and PS-LS stocks) and a central Arctic population (containing C-FB, N-FB, and HB-DS stocks). These populations are moderately differentiated ($F_{ST} = 0.07$), but based on evidence of contemporary movement from assignment tests, are not completely isolated. There was support for maintaining the WJS stock and a combined BB+PS-LS stock, although the latter conclusion is based on a small sample size. Similarly, there was some evidence suggesting separation of the Foxe Basin stocks from the HB-DS but not the N-FB from the C-FB stock. However, given that there are morphological and chemical differences between N-FB and C-FB stocks, there is currently insufficient evidence to support a revision of the current stock designations.

INTRODUCTION

Walruses (*Odobenus rosmarus rosmarus*) occur in Canada from James Bay to Smith Sound and from the Canada–Greenland international boundary in Davis Strait to the longitudinal center of Canada (Fig. 1). Within this range, walruses are subdivided into seven stocks based on summering areas for the purpose of making management decisions that affect walrus and walrus habitat (Stewart 2008). Stock assessments (e.g. Breiwik and York 2009, Lugten 2010) rely on identifying units that can be managed without impact on other units. In the absence of definitive information, it is more precautionary to assume greater subdivision than exists in nature rather than to assume less (Taylor 1997, Taylor and Dizon 1999). However, overly conservative subdivision can lead stock managers to overestimate the risk of stock extirpation, potentially leading to negative effects on resource users.

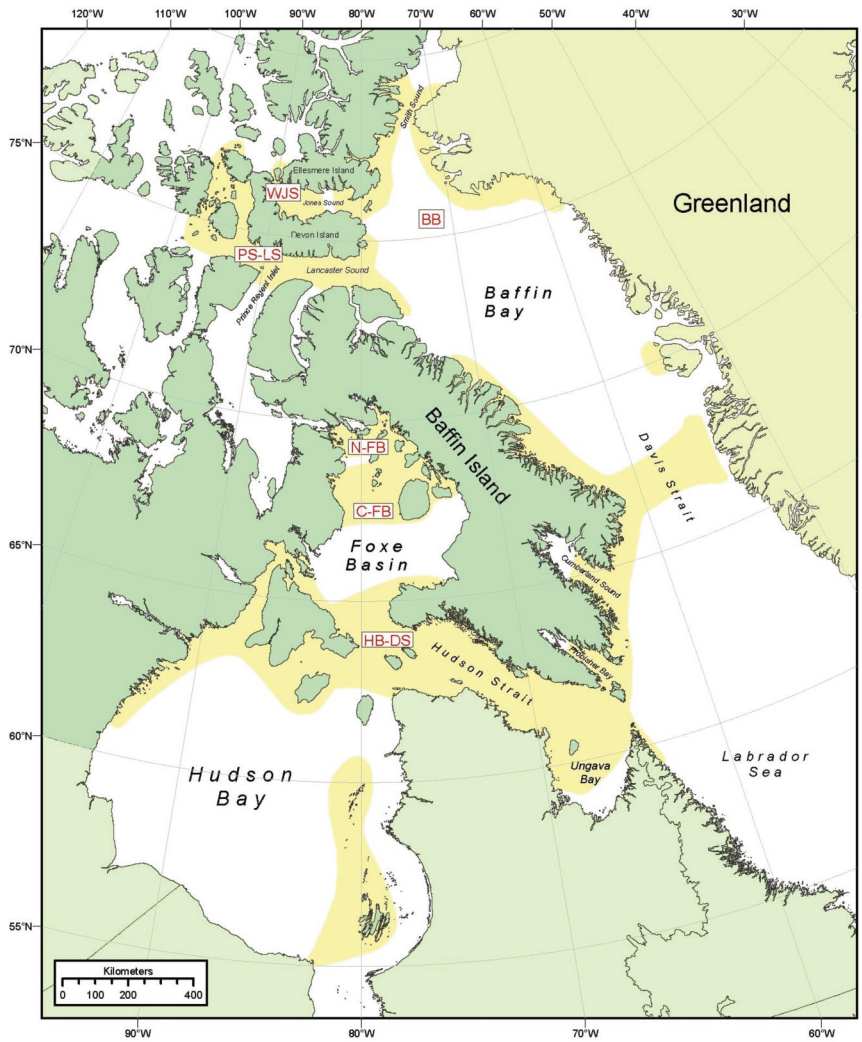

Fig. 1. *Map of putative stocks for walruses in the Canadian Arctic: Western Jones Sound (WJS), Baffin Bay (BB), Penny Strait-Lancaster Sound (PS-LS), North Foxe Basin (N-FB), Central Foxe Basin (C-FB), Hudson Bay Davis Strait (HB-DS), (after Stewart 2008).*

Stewart (2008) hypothesized that there were seven largely isolated stocks of walruses in Canada; Western Jones Sound (WJS), Baffin Bay (BB), Penny Strait–Lancaster Sound (PS-LS), and two stocks in Foxe Basin: the North Foxe Basin (N-FB) and Central Foxe Basin (C-FB). In addition, Stewart (2008) concurred with an earlier review (Born et al. 1995) that the walruses distributed from Northwest Hudson Bay to Davis Strait (HB-DS) were a stock, which probably contained sub-units that were as yet undefined. It was also thought that the South and East Hudson Bay (SE-HB) stock was largely isolated from the other stocks (Stewart 2008). The distribution of the HB-DS stock is now known to extend to West Greenland (Dietz et al. 2014) but there are some genetic differences between Hudson Strait and West Greenland (Andersen et al. 2009, Andersen et al. 2014). Herein, we follow Stewart's (2008) modification of Secor's (2005) definition, recognising a stock is a segment of a population that may be impacted by anthropogenic activities, such that overall population productivity could be affected.

Molecular approaches can offer valuable insights into stock structure and have been used successfully in walruses (Simonsen et al. 1982, Cronin et al. 1994, Andersen et al. 1998, Buchanan et al. 1998, Andersen and Born 2000, Born et al. 2001, de March et al. 2002, Andersen et al. 2009, Andersen et al. 2014). Using microsatellite data, Andersen et al. (2009, Andersen et al. 2014) identified 5 walrus populations surrounding Greenland, which included differentiation

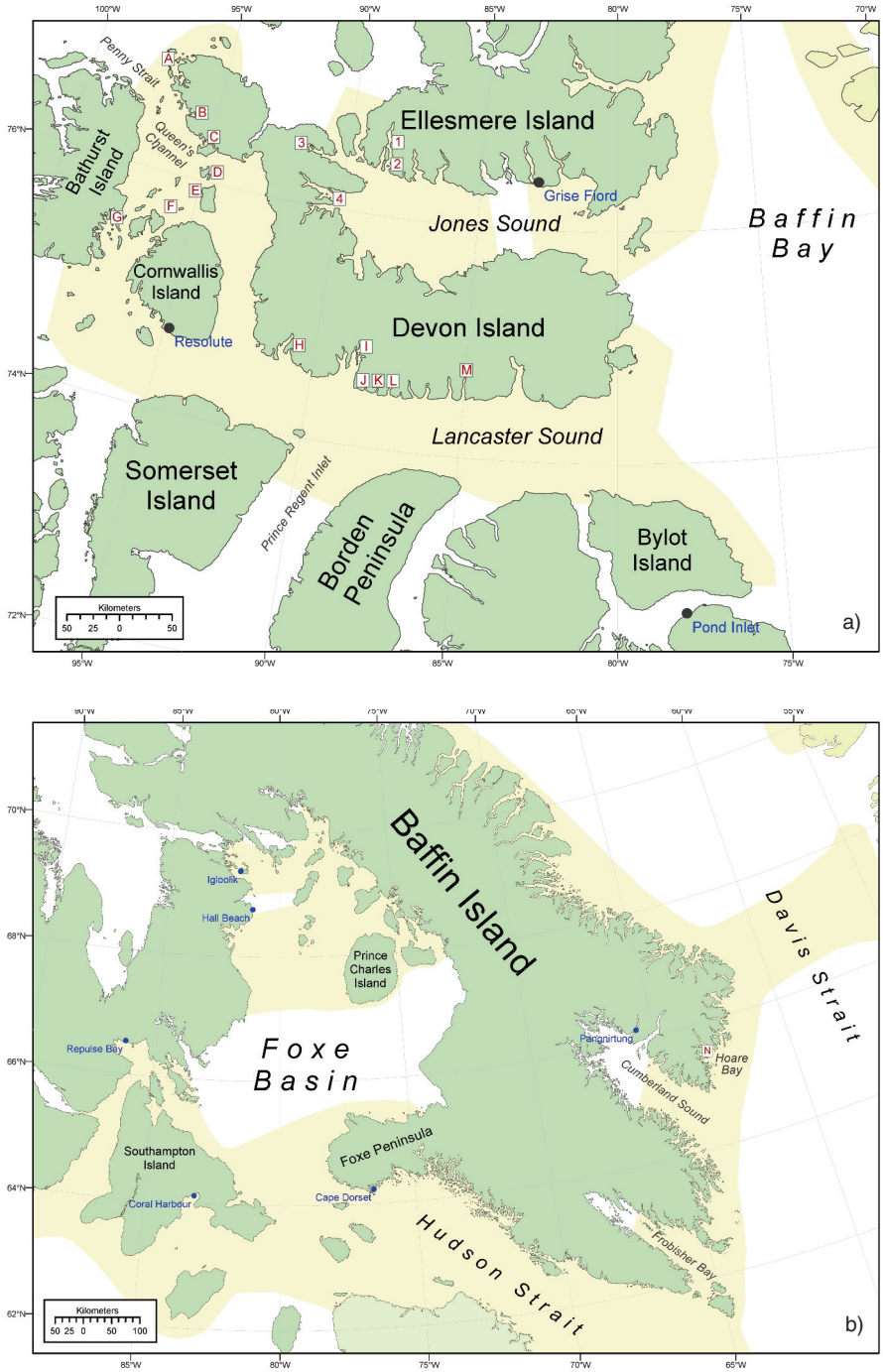

Fig. 2. *Sampling locations and place name used in the text (a) high Arctic locations and (b) central Arctic locations. Site designations are also presented in Appendix I. 1. Mount Borgen; 2. Clement Ugli; 3. Norfolk Inlet; 4. West Channel; A. Village Bay; B. Barrow Harbour; C.Dyer Island; D. Margaret Island E. Ballie- Hamilton Island; F. Houston-Stewart Island; G. Brooman Point; H. Kearney Cove; I. Ryder Inlet; J. Graham Inlet; K. No Name Bay*; L. Blanely Bay; M. Cuming Inlet. (* this tiny bay has neither a local nor an official name [I. Kalluk, Chair, Resolute Bay HTA, pers. comm.; T. Janzen, Hydrographer, Canadian Hydrographic Service, pers. comm.]).*

Fig. 3. *Biopsies were taken using a CO_2 powered dart gun (a) usually on land but also (b) in the water in Hoare Bay (photos by A. MacHutchon (a) and Robert E.A. Stewart (b)).*

between Hudson Strait and West Greenland. Although efforts have been made to identify subdivision within Canadian stocks (Outridge and Stewart 1999, Outridge et al. 2003), a population genetic approach has yet to be applied. Here, we used tissue samples from harvested and biopsied animals to examine the stock structure of walrus populations in Canada using microsatellites. We specifically addressed three questions: 1) Is there genetic differentiation among Stewart's (2008) designated stocks? 2) Is there genetic differentiation, spatially and temporally, within stocks? and 3) What is the rate of genetic exchange between stocks? Following Stewart (2008), we employed Pianka's (1988) definition of a population, an intraspecific group with a higher probability of interbreeding than breeding with members of other groups, and used wintering areas as surrogates for population identification because breeding takes place in winter (Sjare and Stirling 1996). We also adopted Pianka's (1988) definition and use the microsatellite data to examine, indirectly, the validity of using wintering areas to define populations. We have no samples from wintering areas but assume that a panmictic population would indicate that wintering areas do not represent separate populations. Conversely, any structure revealed in summer samples might be matched to the nearest wintering areas and indicate reproductive isolation. By using microsatellites to address these three questions we provide new evidence on stock structure and diversity and discuss the conservation and management implications.

METHODS

Sample collection

Samples were collected at numerous sites in the Canadian north over twenty-five years (Fig. 2; Appendix I). No samples were available from the putative SE-HB stock. Most samples (414/596) were obtained from harvested animals for which the community represents the sampling location. These samples were usually small pieces of muscle that were initially frozen until a subsample could be removed in the lab and transferred to a saturated NaCl in 20% DMSO solution (hereafter DMSO; Amos and Hoelzel 1991). Some samples were collected from live walruses using a biopsy cutter (Acu-Punch®) on individuals that were chemically immobilized for tagging studies (Stewart 2008, Dietz et al. 2014) or from unrestrained walrus (Fig. 3) using a biopsy dart (PneuDart Type P) and a CO_2 powered gun (Dan Inject Model IM and Model JM). In both cases, the biopsy measured about 6 mm in diameter and ≤ 10 mm long. All biopsies were stored in DMSO within a few hours of collection. All samples in DMSO were frozen at -40ºC until DNA was extracted in the lab.

Laboratory analyses

DNA was extracted using the DNeasy™ Blood and Tissue Kit (Qiagen, Inc., Valencia, CA, USA) following the manufacturer's protocol. The extracted DNA was diluted to 10 ng/µl. Eight microsatellite loci from walruses (Buchanan et al. 1998) and three from grey seals (*Halichoerus grypus*; Allen et al. 1995) were amplified in three duplex and five single PCR reactions: 1) Orr3 + Orr11 2) Orr24 3) Orr7 + Orr23 4) Orr9 + Orr16 5) SGPV9 6) Hg3.6 7) Hg6.1 and 8) HgDii. The 15 µl PCR reactions contained double-distilled water, 1.5X PCR buffer, 0.24 µM of each primer, and 0.24 mM of each dNTP. All reactions contained 3 mM MgCl2 except Hg3.6 that had 1.6 mM. We added 0.5 units of Taq polymerase and 20-50 ng of DNA template. One primer in each set was fluorescently labelled (tags: 6-FAM, TET, or HEX). PCR began with an initial 1-minute denaturation at 95°C followed by 33-35 cycles of denaturation, annealing and extension (details provided in Appendix II). The amplified microsatellites were loaded on an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA) with a GS500TAMRA size standard (Applied Biosystems). Microsatellite alleles were detected, scored and manually verified using GENEMAPPER version 4.0 (Applied Biosystems). To assess genotyping error, blind-replicates were included.

Statistical analyses

For each of the six putative stocks (N-FB, C-FB, HB-DS, BB, PS-LS, and WJS; Fig. 1) defined a priori according to Stewart (2008), we quantified genetic diversity as expected (H_E; Nei 1987) and observed heterozygosity (HO) using the microsatellite toolkit (Park 2001). Allelic richness was estimated using the rarefaction method implemented in HP-RARE 1.0 (Kalinowski 2005). Duplicate genotypes were identified and removed (but see detection of migrants below). The software Micro-Checker (Van Oosterhout et al. 2004) was used to identify potential null alleles. Deviation from Hardy-Weinberg Equilibrium (HWE) was tested using Genepop 4.0 (Rousset 2008) under the alternative hypothesis of heterozygote deficiency. Global tests of HWE for each stock and locus were also performed. We used FSTAT 2.9.3 (Goudet 1995) to test for linkage disequilibrium, with significance assessed by 1000 permutations. We also tested for homogeneity of allele distributions for all pairs of stocks using the probability test (Raymond and Rousset 1995) implemented in Genepop. Significance of P values were based on multiple comparisons (i.e. Bonferroni Correction; Rice 1989)

Microsatellite Analyzer (MSA) 4.05 (Dierenger et al. 2003) was used to calculate Nei's (1972) genetic distance (D_S) between putative stocks. Wright's fixation indices for genetic differentiation (F_{ST}) and inbreeding (F_{IS}) within stocks were also estimated using Weir and Cockerham's (1984) unbiased estimators in FSTAT. Significance was tested using 1,000 permutations. We

then assessed population genetic structure independent of sampling area using the Bayesian assignment software STRUCTURE 2.2 (Pritchard et al. 2000). An admixed model with correlated allele frequencies (Falush et al. 2003) was employed. Five independent runs from $K = 1$ to $K = 10$ were performed using 1,000,000 iterations with the first 25% removed as a burn in. The Ln P(D) and ΔK method of Evanno et al. (2005) was used to identify the primary genetic clusters in the data. Individuals were then assigned to each genetic cluster based on their highest percentage membership (q) calculated from the five runs using the full search in CLUMPP 1.1.1 (Jakobsson and Rosenberg 2007). When clusters were identified, STRUCTURE was run again to identify any within cluster substructuring.

We examined genetic spatial autocorrelation between walruses according to haul-out site using the program SPAGEDI 1.3 (Hardy and Vekemans 2002). We estimated a relationship coefficient (Moran's I) between all pairs of individuals and calculated the distance between adjacent sampling sites according to sea-kilometers. Spatial autocorrelation was assessed at 100 km distance classes because the minimal average distance separating stocks was 70 km and this avoided lumping stocks together. Significance of the linear regression slope and the standard error were calculated by 1,000 permutations and a jackknifing procedure, respectively. We had sufficient samples (i.e., 12 to 48 animals per sampling year) from Foxe Basin to examine temporal changes in allele frequencies in the form of "isolation-by-time" (Hendry and Day 2005; Demandt 2010), which is the association between genetic similarity and number of years between sampling events. From 1983 to 2007, twelve different annual sampling events took place and were included in our data set (Appendix I). We constructed two matrices: i) FST between all sampling events and ii) number of years separating each sampling year. The relationship between matrices was evaluated in R2.9.2 (http://www.r-project.org/) using a Mantel Test under 1,000 permutations implemented in the Ecodist library (Goslee and Urban 2007). Because of our minimal knowledge of stock sizes and temporal trends (COSEWIC 2006), we also measured HO over the sampled years using a simple linear model. These analyses allow for monitoring of gene frequencies over time, permitting us to gauge the influence of gene flow, genetic drift and selection on a population.

We used three approaches to genetically identify movement between stocks. We first assessed whether any of the biopsied animals were subsequently sampled in a different stock. To do this, we calculated the probability of identity using GenAlEx 6.2 (Peakall and Smouse 2006) and screened the data for duplicate genotypes (i.e. zero mismatches). The second approach used the STRUCTURE and GeneClass 2.0 (Piry et al. 2004) assignment probabilities. Any individual assigned to a cluster by STRUCTURE with a q>0.80 that was not common within their population (< 20% of assigned individuals) was considered cross-assigned and indicative of contemporary movement between stocks. Finally, GeneClass 2.0 was used to identify first generation migrants. We used a Bayesian method (Rannala and Mountain 1997) with Monte-Carlo resampling and 1,000 simulated individuals (Paetkau et al. 2004). We then compared the likelihood of an individual being from the sampled stock, relative to all the other stocks (Paetkau et al. 2004) to detect migration events.

Table 1. Number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), Wright's inbreeding coefficient (F_{IS}), and evidence of a null allele for each marker used in this study

Locus	A	H_O	H_E	F_{IS}	Null
Orr16	12	0.78	0.83	0.06	No
Orr23	17	0.73	0.88	0.16	Yes
Orr7	14	0.85	0.87	0.02	No
Orr9	6	0.63	0.68	0.07	No
Orr11	7	0.58	0.61	0.04	No
Orr24	10	0.73	0.78	0.06	No
Orr3	8	0.68	0.71	0.04	No
HG6.1	4	0.41	0.44	0.06	No
HGDii	7	0.29	0.32	0.08	No
HG3.6	3	0.26	0.27	0.02	No
SPVg9	4	0.62	0.65	0.05	No

Table 2. Estimates of genetic variability across Canadian walrus stocks: Number of sampled individuals (*N*), observed heterozygosity (H_O), expected heterozygosity (H_E), and allelic richness estimated by rarefaction (AR), and Wright's inbreeding coefficient (F_{IS}). No FIS differed significantly from zero (p>0.05) except WJS (p=0.02)

Stock	*N*	H_O	H_E	AR	F_{IS}
Baffin Bay	15	0.57	0.58	3.8	0.03
Central Foxe Basin	139	0.60	0.60	4.1	0.01
Northern Foxe Basin	237	0.59	0.60	4.1	0.02
Hudson Bay - Davis Strait	76	0.61	0.60	4.1	0.00
Penny Strait - Lancaster Sound	121	0.56	0.57	3.8	0.03
West Jones Sound	35	0.53	0.57	3.6	0.07

RESULTS

We obtained 724 samples of which 596 individuals were genotyped at a minimum of 9 loci. A subsample of these 596 individuals contained 41 blind-replicates that were included and genotyped with 100% accuracy. After reviewing 11 loci for genotyping problems, Orr23 was identified as potentially having null alleles (Table 1) and removed from the analyses. The final data set consisted of 596 individuals genotyped at 10 loci, which was >96% complete. All but the WJS stock showed FIS values around zero (average 0.03), while rarified alleles and heterozygosity measures were similar among all stocks (Table 2). Two markers (Orr11 and HG3.6) were each out of HWE in one of the six stocks, but were retained in the data set (Table 3). There was no evidence of linkage disequilibrium. Of the 150 loci paired-stock combinations, 90 had allele frequency distributions that differed after Bonferroni correction suggesting population differentiation (Table 3).

Global F_{ST} was 0.06 and pair-wise F_{ST} and D_S varied among stocks (Table 4). F_{ST} pair-wise comparisons were not significant between N-FB and C-FB, or between PS-PL and BB stocks, but all others were significant at p=0.05. Bayesian analysis resolved a single subdivision at K=2 based on Ln P(D) and ΔK (K=1 the average Ln P(D) was -18,487; at K=2 it was -17,640 and at K=3 it was -17,690). These clusters had no additional structure (i.e., all subsequent Ln P(D)'s were further away from zero). The two inferred clusters had the stock designations nested within them, with cluster 1 highly represented in the C-FB, N-FB and HB-DS stocks, and cluster 2, highly represented in the BB, PS-LS and WJS stocks (Table 5). We refer to these clusters as the central Arctic (cluster 1) and high Arctic (cluster 2) populations. The two clusters were significantly differentiated (F_{ST}=0.07 and D_S=0.11). At the individual level, 125 of 139 C-FB walrus unambiguously assigned (i.e. q>0.80) to the central Arctic population, as did 215 of 237 N-FB and 49 of 76 HB-DS individuals. In the remaining stocks, 14 of 15 BB individuals, 108 of 121 PS-LS individuals, and 34 of 36 WJS individuals were unambiguously assigned (Fig. 4) to the high Arctic population.

Table 3. Tests of Hardy-Weinberg Equilibrium, linkage disequilibrium, and allelic distribution across the six walrus stocks. Three significance levels are shown, with the expected number of Type I error if the null hypothesis is correct given each alpha value. Square brackets denote the Bonferroni-corrected alpha value. (Obs. = Observed, Exp. = Expected)

	Individual HW		Global HW (stocks)		Global HW (loci)		Linkage disequilibrium		Allele distributions	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
P < 0.05	8	3	2	0.3	4	0.50	34	13.5	90	7.5
P < 0.01	4	0.6	2	0.06	2	0.10	12	2.7	81	1.5
Bonferroni	2	[0.001]	2	[0.01]	2	[0.005]	0	[0.0002]	65	[0.0003]

Table 4. Genetic distances between walrus stocks. Below the diagonal are FST values with insignificant values denoted by NS. (corrected for multiple tests p=0.003). Above the diagonal is Nei's DS.

	BB	C-FB	N-FB	HB-DS	PS-LS	WJS
Baffin Bay (BB)	-	0.12	0.12	0.10	0.02	0.03
Central Foxe Basin (C-FB)	0.08	-	0.00	0.10	0.11	0.13
Northern Foxe Basin (N-FB)	0.08	0.00NS	-	0.01	0.11	0.13
Hudson Bay - Davis Strait (HB-DS)	0.07	0.01	0.01	-	0.09	0.10
Penny Strait - Lancaster Sound (PS-LS)	0.01NS	0.07	0.07	0.06	-	0.04
West Jones Sound (WJS)	0.02	0.08	0.09	0.07	0.03	-

We detected a negative genetic spatial autocorrelation ($p<0.01$), although the magnitude varied between southern and northern clusters (Fig. 5). Temporal analysis within FB, averaging 31 individuals per sampling event, showed no differentiation in 12 sampling events across 25 years (F_{ST} from –0.01 to 0.02, all p's>0.05). There was no isolation-by-time pattern (mantel r=0.03, p=0.40) and the temporal H_O ranged from 0.56 to 0.65 with no discernable trend in our linear model (p=0.66).

We calculated the probability of identity at 10 loci to be 8.0×10^{-08}, which suggested that duplicate genotypes are likely the same individual rather than two individuals with the same genotype. Six pairs of individuals sampled in different years had identical genotypes. Four (1 female WJS, 3 males, PS-LS) of the six duplicates were recaptured at the same haul-out 2-3 years later. Two duplicates (males) were resampled at different haul-outs (within PS-LS), roughly 100 and 250 sea-kilometers apart, two years later. A small proportion of samples were assigned to the genetic cluster not common in their stock of origin. Twelve individuals sampled in Foxe Basin (4 from Hall Beach; 2 from Igloolik) and Hudson Bay–Davis Strait (2 each from Cape Dorset, Coral Harbour and Hoare Bay) were strongly (q>0.80) assigned to the high Arctic population. In the high Arctic sample, two individuals (1 each from Cumming Inlet and Pond Inlet) were assigned (q>0.80) to the central Arctic population. GeneClass 2.0 classified five samples as first generation migrants. Three migrants were between clusters (two of which were identified using the STRUCTURE approach), while the other two were within cluster migrants.

Table 5. Genetic assignment matrix. Average assignment and number of individuals strongly assigned (q>0.80) from each stock to the central Arctic and northern-Arctic genetic clusters inferred from STRUCTURE.

		Central Arctic Cluster		Northern Arctic Cluster	
	Sample Size	Average q	N, q>0.8	Average q	N, q>0.8
Baffin Bay (BB)	15	0.05	0	0.95	14
Central Foxe Basin (C-FB)	139	0.91	125	0.09	4
Northern Foxe Basin (N-FB)	237	0.92	215	0.08	2
Hudson Bay–Davis Strait (HB-DS)	76	0.78	49	0.22	6
Penny Strait–Lancaster Sound (PS-LS)	121	0.08	5	0.92	108
West Jones Sound (WJS)	35	0.06	0	0.94	33

DISCUSSION

Walruses in Arctic Canada are divided into two large genetic groups: the central Arctic population that contains the N-FB, C-FB, and HB-DS stocks, and the high Arctic population that contains the BB, PS-LS, and WJS stocks (Fig. 4). This is the uppermost hierarchical break in the population and no additional subdivisions were supported by the ΔK method. There were no data available for the SE-HB stock, and hence this stock was not included in this assessment. The levels of between-stock differentiation are consistent with the STRUCTURE-inferred clusters, with comparisons between central and high Arctic stocks showing the highest differentiation (Table 4). While comparisons among stocks within the central and high Arctic groups were often statistically significant (Table 4), this is likely due to sample size and we regarded these comparisons as showing minimal biologically significant genetic differentiation. There appears to be some contemporary movement between populations as indicated by the cross-assignments and migration patterns. Compared to walrus in Greenland that were examined using many of the same microsatellite markers (Andersen and Born 2000, Andersen et al. 2009, Andersen et al. 2014), the Canadian Arctic stocks showed slightly lower levels of genetic diversity in terms of both heterozygosity and number of alleles. However, in contrast to Andersen and Born (2000)

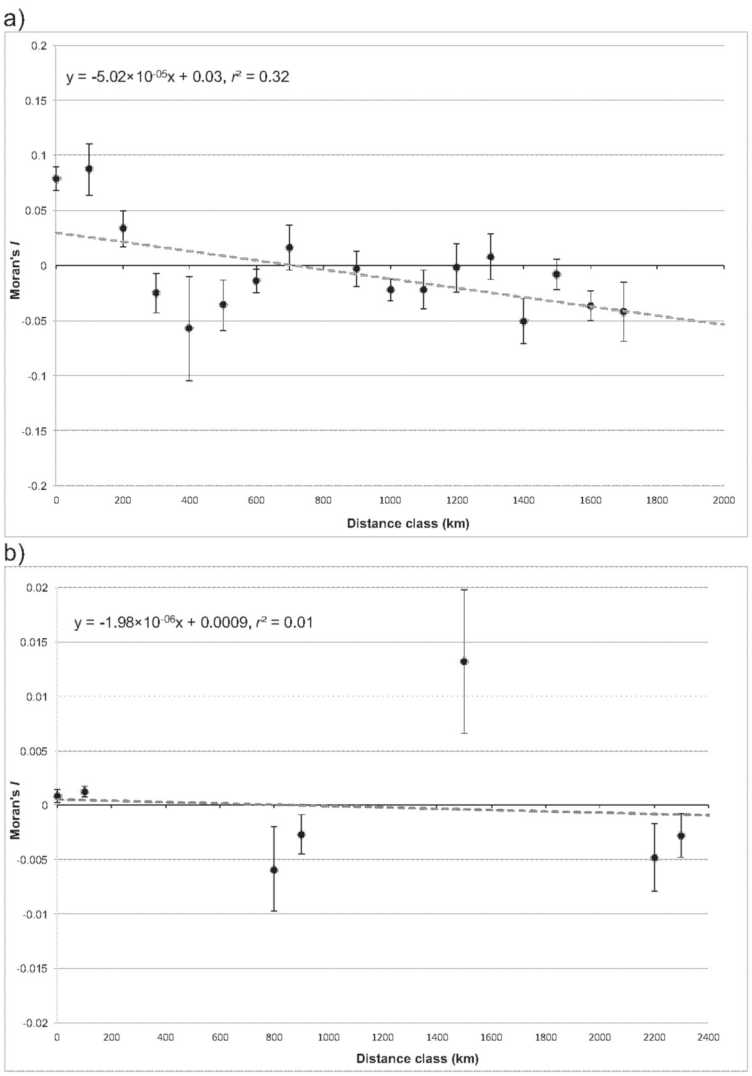

Fig. 4.
Spatial auto-correlation among individuals sampled at haul-out sites, based on Moran's I, for walruses in the a) high Arctic, and b) central Arctic clusters inferred from STRUCTURE. The equation for the line and correlation coefficient are provided.

and Andersen et al. (2009, 2014), levels of differentiation were higher in Canadian Arctic walrus and the low F_{IS} values suggest minimal inbreeding within Canadian stocks. Overall, our analysis of walrus in Arctic Canada suggests the designated stocks are genetically diverse and are units nested within larger high and central Arctic populations.

Assignment analysis placed 88.2 % and 86.7% of individuals strongly (q>0.80) in the high Arctic and central Arctic groups respectively. Two individuals (1.1%) sampled in high Arctic stocks were strongly assigned to the central Arctic genetic cluster. Both were sampled in eastern Lancaster Sound (PS-LS). Twelve individuals (2.7%) sampled in the central Arctic area were more likely to be high Arctic genotypes. While the precise number of cross-assignments will be sensitive to the assignment criterion (i.e. q=0.80 applied here) and search algorithm, this north-south difference in cross-assignments hints that movement of walrus from the high Arctic population to the central Arctic population might be more prevalent.

Genetic differentiation among stocks

The central Arctic population includes two stocks in Foxe Basin plus the Hudson Bay–Davis Strait stock. Within Foxe Basin, there appears to be genetic continuity despite spatial variation in Pb-isotopes and trace elements detected in landed catches at Igloolik and Hall Beach (Outridge and Stewart 1999) that would suggest these are two stocks with different life styles and different exposures to harvesting. Local Inuit also distinguish between two types of walrus in Foxe Basin on the basis of size, colour and distribution (DFO 2002). The lack of genetic differentia-

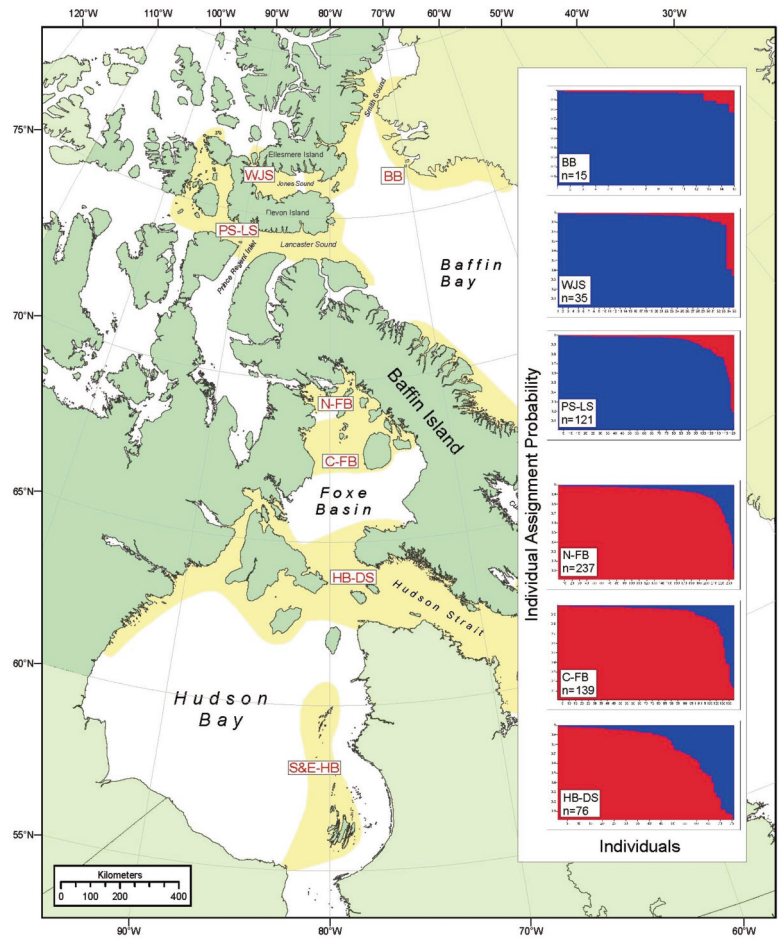

Fig. 5.
Map of putative stocks, with individual assignments to shown groups according to stocks. Western Jones Sound (WJS), Baffin Bay (BB), Penny Strait–Lancaster Sound (PSLS), North Foxe Basin (N-FB), Central Foxe.

tion between FB and HB-DS stocks (Table 4) also contrasts with the Born et al. (1995) and the Stewart (2008) hypothesis that FB was largely isolated from all other stocks. Lead isotope ratios suggested that the majority of walrus in FB reside in a different geochemical environment and constitute a separate stock (Outridge and Stewart 1999). In that study, approximately 20% of the samples from Hall Beach were statistical outliers which, upon further investigation of isotope ratios in individual growth layers in the teeth (Stewart et al. 2003), indicated the presence of both immigrants, and the departure and return of mature males. Stewart et al. (2003) likened the latter behaviour to the roving males of primatology (Suzuki et al. 1998), whereby males may breed in a number of different areas, and suggested that male mediated genetic exchange (see also Andersen and Born 2000) between FB and the HB-DS and SE-HB Bay stocks is possible. The statistical outliers (Outridge and Stewart 1999) included three nursing young that likely represented the movement of pregnant females from other areas. Although long-range movements of small calves were discounted in earlier studies, it is now apparent that females with newborn calves can cross Davis Strait from Greenland to Baffin Island (Dietz et al. 2014). An exchange of 20% (Outridge and Stewart 1999) is likely more than adequate to maintain the genetic homogeneity reported here and the earlier isotopic data now appear consistent with a single interbreeding population including the C-FB, N-FB and HB-DS stocks.

In a preliminary population genetic study, de March et al. (2002) found no differences between BB and PS-LS stocks but did find differences between these stocks and FB. Based in part on those preliminary genetic studies, isotope and distribution data, Stewart (2008) tentatively suggested PS-LS and BB walrus might represent two stocks of a single population but the rationale for separating these two stocks rested solely on limited Pb-isotope data. Stewart (2008) also noted the possible separation of WJS and a population near Dundas Island in PS-LS based on the observed distribution and the lack of tag movement between the two areas. Our results provide greater evidence for one population of walrus encompassing the BB, WJS and PS-LS stocks. The degree of exchange of breeding animals between over-wintering areas in the western part of the range, e.g. Dundas Island polynya and the mouths of Jones and Lancaster sounds in the east is unknown, but given the minimal genetic differentiation, they are likely genetically contiguous. The proposed separation of a WJS population by Stewart (2008) is not supported by STRUCTURE analysis, although the sample size of BB walrus was small (n=15). Conversely, while the level of differentiation between WJS and PS-LS was low and non-significant (F_{ST}=0.01, $p>0.05$), a small difference between WJS and BB was detected (F_{ST}=0.02, $p<0.05$). We also detected some evidence of genetic spatial autocorrelation, most notably in the high Arctic, which indicates that haul-out sites located within a few hundred kilometres within stocks are slightly more genetically similar than ones farther apart (Fig. 4). The level of autocorrelation suggests that most of our sampled individuals at the same haul-out were not close relatives (i.e. siblings, parent-offspring). Ultimately, the apparent genetic differentiation between WJS and BB+PS-LS stocks requires further investigation.

There are two important caveats to a population genetic analysis of stock structure to note. First, it takes relatively few immigrants per generation to maintain a signal of genetic connectivity. A few migrant bulls, or a few breeding forays per generation, can prevent genetic differentiation from accruing between demographically distinct stocks. Also, the observed genetic differentiation reflects historic conditions and may take longer to accrue than life-style indicators (Swain et al. 2005, Waldman 2005, Wirgin and Waldman 2005, Stewart 2008). Additionally, lack of high genetic differentiation does not reject stock designations based on other markers (Outridge and Stewart 1999, Innes et al. 2002, Outridge et al. 2003, Campana 2005). Secondly, following the retreat of the Laurentide ice-sheet, walruses were thought to have rapidly recolonized northern waters, reaching the Canadian Arctic only ~10,000 years ago (Dyke et al. 1999). The low differentiation within the northern and central Arctic groups could simply reflect this rapid expansion, accompanied by the high mobility of walruses (and their relatively large population sizes).

Interestingly, this overarching split might be reflective of multiple refugia during the last glacial maximum or multiple recolonization routes. This hypothesis needs to be explored further with additional molecular markers. Overall, our study suggests a low long-term rate of genetic exchange between the central and high Arctic populations.

Genetic differentiation within stocks

Although between stock analyses can inform range-wide management and stock designations, local and temporal analyses allow for monitoring of gene frequencies over time. This approach allows researchers to gauge the influence of gene flow, genetic drift and selection on a population, all of which are drivers of evolutionary change (Demandt 2010). Evaluating such processes is critical for assessing a population's evolutionary potential. In this study, we only had sufficient data from the FB stock (i.e. average of 31 individuals per sampling year) to assess temporal changes. The FB stock is believed to have at least 2700 animals, but no temporal trend in abundance is known (COSEWIC 2006). In the past 50 years, the summer distribution has shifted (Anders 1966, Crowe 1969, Beaubier 1970, Brody 1976, Orr et al. 1986) and there has been a marked increase in boat traffic and hunting pressure (COSEWIC 2006). However, we found no genetic differences either temporally or spatially between walrus landed at Hall Beach and Igloolik within the FB stock, suggesting the stock is relatively stable genetically.

FINAL CONCLUSIONS AND CONSIDERATIONS

A stock may be defined by geographic distribution or differences in life histories (Outridge and Stewart 1999, Innes et al. 2002, Outridge et al. 2003, Campana 2005), but the presence of genetic differences likely indicates a longer separation. Our analysis of Arctic walruses from the Canadian north suggests a well-established genetic subdivision between a high Arctic population with two moderately genetically differentiated stocks (BB+PS-LS and WJS) and a central Arctic population with two moderately genetically differentiated stocks (N-FB+C-FB and HB-DS). The rationale for separating the BB and PS-LS stocks is tenuous given essentially zero genetic differentiation and requires more investigation; however, the non-genetic differences between N-FB and C-FB (Stewart 2008) argue in favour of them having separate stock designations. Given the precautionary mandate of stock designations (Taylor 1997, Taylor and Dizon 1999), currently there is insufficient evidence to revise the stock structure proposed by Stewart (2008). We also found that wintering areas are a poor surrogate for populations. Spatial autocorrelation within each cluster, most notably the northern Arctic cluster, suggests some degree of geographically restricted gene flow within clusters. The extent to which gene flow has been restricted by winter ice conditions is unknown but ice conditions are changing and it is reasonable to expect these relationships may decay or shift over time (see also Kelly 2001, Petersen et al. 2010). In addition, the genetic relationship of walruses in southern Hudson Bay to the six stocks examined here remains unknown. Further genetic monitoring and sampling will enable us to assess the adaptive potential of walrus, as well the impact of changing sea ice on population structure and diversity.

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