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Genetic signals of historic and recent migration between sub-populations of Atlantic walrus (*Odobenus rosmarus rosmarus*) west and east of Greenland

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Abstract

Defining sub-populations and determining migration rates between them is crucial for sound management and conservation. Defining sub-populations and determining migration rates also applies to Atlantic walruses (*Odobenus rosmarus rosmarus*), which are still exploited despite low population levels in some cases and lack of information on abundance in others. Analysis of muscle and skin tissues using 11 nuclear microsatellite markers from a total of 297 Atlantic walruses from Hudson Strait (Hudson Strait) across West, Northwest and East Greenland (W Greenland, NW Greenland, E Greenland) to Svalbard and Franz Josef Land was undertaken to determine the number of sub-populations, ancestral origin and the contemporary rates and directions of migration (gene flow) between the various areas. The study indicated the existence of five sub-populations in Hudson Strait, W Greenland, NW Greenland, E Greenland and Franz Josef Land-Svalbard. Identification of the Hudson Strait sub-population was novel; although differences between animals here and in W Greenland were small, the differences were statistically significant and indicated that walruses in Hudson Strait could be a source of walruses for the W Greenland sub-population. The direction of migration (Hudson Strait to W Greenland) suggested a genetic signal from a historical large-scale counter-clockwise perennial migration pattern in the Baffin Bay region. The study provides essential information on population sub-structuring that is a prerequisite for management of Atlantic walruses at sustainable levels.

Introduction

Conservation concerns

Throughout their range Atlantic walruses (*Odobenus rosmarus rosmarus*) have been heavily exploited causing reductions in range and number compared to recent historical times (Born et al. 1995). In many parts of Canada and Greenland estimates of abundance do not exist or are merely educated guesswork. Several populations are thought to number less than a few thousand individuals (NAMMCO 2006, COSEWIC 2006).

Currently, Atlantic walruses are hunted by Inuit in Canada throughout South and East Hudson Bay-Hudson Strait and in West, North West and East Greenland (W Greenland, NW Greenland, E Greenland). Exploitation rates in W Greenland and NW Greenland are thought to be unsustainable (Born et al. 1994, 1995, Anon. 1995, Witting & Born 2005, NAMMCO 2006, COSEWIC 2006), emphasising the importance of sub-population recognition and identification of migration rates (gene flow) and their directions.

In 2006, Atlantic walruses in Canada, including two shared sub-populations in W Greenland and NW Greenland, were designated “Species of special concern” by COSEWIC (Committee on the Status of Endangered Wildlife in Canada). The assessment concluded that the species is near to qualifying for threatened status and requires an effective plan for management of the exploitation (COSEWIC 2006). COSEWIC defines a “Species of special concern” as a “species that might become threatened or endangered” based on a combination of biological and other identified threats; “Threatened” implies that a species is likely to become “Endangered” facing imminent extirpation or extinction if limiting factors are not moderated or removed (Ibid.).

Historically, walruses were heavily exploited in the Svalbard and Franz Josef Land areas, causing a serious decline in numbers. As a result, walruses were completely protected in these areas in 1952 and 1956, respectively (cf. Gjertz & Wiig 1994, Born et al. 1995). However, in recent years there have been signs of recovery in this population, and a survey in 2006 suggested ca. 2600 walruses in the Svalbard area alone (NAMMCO 2006, Lydersen et al. 2008).

To ensure sustainability, it is common practice to manage large Arctic marine mammals at the sub-population level (e.g. polar bears, *Ursus maritimus*, Aars et al. 2006; beluga, *Delphinapterus leucas*, Alvares-Flores & Heide-Jørgensen 2004; narwhal, *Monodon monoceros*, Anon. 2005). Information on the geographical distribution, genetic identity and number of sub-populations and rates of exchange of individuals between them is therefore crucial.

Recent and historical migration patterns between populations are important factors in shaping the contemporary genetic population structure (Ramstad et al. 2004). The existence of several sub-populations or stocks of Atlantic walruses have been suggested (Born et al. 1995, NAMMCO 1995, 2006). However, in several cases knowledge about population sub-structure is insufficient to assess them separately (COSEWIC 2006). This study analyses the population structure and migration between groups of walruses in the Hudson Strait area, W Greenland, NW Greenland, E Greenland, Svalbard and Franz Josef Land (Table 1, Fig. 1).

Background

To understand the background for the study, it is important to describe some life history characteristics of walruses and the ecological situation in the Baffin Bay region where walruses are exploited by Canadian and Greenlandic Inuit.

Life-history characteristics

Walruses have life history traits typical of large mammals. They are relatively long lived, grow slowly and show delayed onset of sexual maturity. They produce few offspring per cycle but invest heavily in each (Fay 1982). Average age at attainment of sexual maturity (first ovulation) in female Atlantic walruses in Greenland is around 6 years, so first births occur at ca. 7 years of age (Born 2001). The generation time (defined as the mean age of mothers producing offspring in a given year) in walrus is close to 15 years (i.e. between sexual maturity and age at reproductive senescence 25-30 years, Fay 1982). The reproductive cycle is basically triennial; adult females give birth every third year, usually to a single calf (Born 2001, Fay 1982). Atlantic male walruses become sexually mature between 7 and 17 years of age (average: 11 years) (Born 2003). However, Pacific walruses (*O. r. divergens*) usually do not participate in mating until physically mature at 13-16 years (Fay 1982), and the same is probably the case in Atlantic walruses.

Atlantic walruses occupy a large geographical range but a relatively narrow ecological niche. They require shallow waters with bottom substrates that support a productive bivalve community, which retain open water above feeding areas in winter, and suitable ice or land nearby upon which to haul out (COSEWIC 2006). Walruses show a high degree of fidelity to such foraging sites (e.g. Born et al. 1997). Female walruses are found in polynyas or similar geographically restricted open water areas surrounded by ice during the mating season (e.g. Sjare & Stirling 1996) that peaks in January-April (Fay 1982, Fay et al. 1984, Born 2001). The generally sedentary nature of walruses during winter and the inherent gregariousness of females appear to have been important factors influencing the evolution of the species' social behavior and mating system (Sjare & Stirling 1996). These factors also are likely driving forces behind the establishment of genetically different sub-populations of Atlantic walruses.

Possible migration patterns between areas

Historically, walruses in the Baffin Bay region apparently were more numerous and were more widely distributed than at present (Freuchen 1921, Vibe 1950). It was suggested that walruses made large-scale, counter-clockwise perennial migrations in the Baffin Bay region between Canada (i.e. East Ellesmere Island and East Baffin Island and the Hudson Strait areas) and W and NW Greenland (Freuchen 1921, Vibe 1950). This suggestion was based on timing of the presence in near-shore areas as well in relation to timing of ice breakup in different areas. The timing of catches indicated that when walruses left their wintering grounds in Central W Greenland in spring, they migrated north along the western coast of Greenland to the North Water area (i.e. northern Baffin Bay-Smith Sound), where a portion of the population had overwintered. This northward migration in spring was possible because ice break-up occurs relatively early along the western coast of Greenland due to the influx of relatively warm water from the Irminger Current in the south (see for example Born & Böcher 2001). During fall the formation of sea ice develops from west towards the east (i.e. from Baffin Island towards western Greenland). Hence, the appearance of walruses along eastern Baffin Island in late summer and their reappearance in fall in Central West Greenland supported 1) the theory of a counter-clock overall migration pattern in the Baffin Bay area, and 2) that this migration was determined by the timing of ice break-up and ice formation in the region (Freuchen 1921, Vibe 1950). However, over-exploitation seemed to cause walrus numbers to decline throughout their range (Born et al. 1995 and references therein). Mansfield (1973) noted the absence of reports of any large scale migrations in the Baffin Bay region during the second half of the 20th Century. He hypothesised that the decline in numbers, and the innate dependence of walruses on sessile food resources in localised shallow coastal waters, had made walruses more sedentary in the Baffin Bay-Davis Strait region (Vibe 1950, Fay 1982).

Objectives

The present study aimed to provide information on population sub-structure necessary for management of Atlantic walruses at sustainable levels. The objectives were: (1) To estimate the number of sub-populations that are represented in a combined sample from parts of the range of Atlantic walrus, (2) to analyse the historical and contemporary migration patterns within and between sub-populations, and 3) to determine the historical and contemporary effective population size. Objective 2 evaluates the hypotheses of enhanced sedentary contemporary behaviour, and the presumed historical counter-clock-wise migration, in the Baffin Bay region between eastern Canada and western Greenland, and Objective 3 could indicate whether the different sub-populations had experienced a population bottleneck.

Material and methods

Samples

Samples of tongue tissue from 58 Atlantic walruses from Hudson Strait (Fig. 1) (12 from eastern Hudson Strait and 54 from western Huston Strait) were collected by Canadian Inuit from their subsistence catch during 1998-2000. The samples were frozen at –20^{o}C until analysed in the laboratory of National Environmental Research Institute (Silkeborg, Denmark). The samples had information on site of the catch and/or name of the community to which the sample was delivered (for regions, stocks, sample size and abbreviations see Table 1). The samples from Canada are referred to as Hudson Strait in the following as there was no significant genetic difference between the two samples (data not shown).

Walruses from central W Greenland, NW Greenland, E Greenland, Svalbard and Franz Josef Land have been previously analysed genetically (Andersen et al. 1998, Andersen & Born 2000, Born et al. 2001). The previous E Greenland sample of 28 individuals was supplemented with a further 48 individuals. These specimens were not genetically different from the previously analysed E Greenland samples (F_{ST}= 0.003, 95%CI: -0.001-0.006; FSTAT, Goudet 1995) based on the eleven microsatellite markers used in the present study (*Hg 6.1, ORR7, ORR3, SGPV9, HGDii, ORR24, ORR23, Igf-1, ORR11,ORR9, ORR16* (Andersen et al. 1998)) and hence all E Greenland data were pooled (Table 1).

All analyses were conducted using N, the number of individuals actually sampled on the different sampling locations, except for the estimation of N_e where N** was used (see later) (Table 1).

DNA was extracted from the Hudson Strait and the recent E Greenland samples using a modified CTAB-buffer method (Milligan 1992) including proteinase K. The 11 microsatellite primers used and their PCR conditions are given in Andersen et al. (1998) and Andersen & Born (2000). PCR products were electrophoresed using an ABI PRISM 377 DNA sequencer and subsequently genotyped.

Sex determination

The gender of the samples from Hudson Strait, E Greenland and Franz Josef Land lacking phenotypic information on sex was determined using a sex-specific primer set for walrus (Fischbach et al. 2008) (for numbers of males, M, and females, F, see Table 1).

Data analyses

Genetic variation was estimated as observed and expected heterozygosity and allele richness in FSTAT (Goudet 1995). Tests for goodness of fit to Hardy-Weinberg expectations (HWE) were performed in GENEPOP (Raymond & Rousset 1995). Significance levels were computed using Fisher's exact test (Guo & Thompsen 1992). Tests for linkage disequilibrium between all pairs of loci were conducted for separate samples and analysed in FSTAT (Goudet 1995).

Objective 1-Estimation of the number of sub-populations

The present study re-analyzes the walrus data in Andersen et al. (1998), Andersen & Born (2000) and Born et al. (2001) (i.e. NW Greenland, W Greenland, E Greenland plus new data, Svalbard and Franz Josef Land), supplemented with the Hudson Strait sample. The degree of population differentiation was analysed using unbiased F_{ST} statistics (Weir & Cockerham 1984) performed in FSTAT (Goudet 1995) and based on sampling locations. Furthermore, we pooled samples from all areas and evaluated assumptions that they originated from two to eight sub-populations without using prior information of sample origin. The most likely number of sub-populations was estimated using a Bayesian clustering method that groups individuals so as to minimise Hardy-Weinberg disequilibrium and gametic phase disequilibrium within groups, as implemented in STRUCTURE (Pritchard et al. 2000). We used 1,000,000 iterations of a Markov Chain Monte Carlo (MCMC) search algorithm with burn-in of 100,000 and two runs. For each number of postulated populations (k), STRUCTURE finds the optimal partitioning of individuals into k groups and, for each individual, estimates the proportion of its genes (q_k) that are derived from each of the k sources.

Objective 2- Estimation of historical and contemporary migration patterns

The historical migration pattern and long-term effective population size was estimated in MIGRATE (MIGRATE 2.1.0 http://popgen.csit.fsu.edu/migrate.download.html) (Beerli 2006, Beerli & Felsenstein 2001) using the likelihood approach. The method assumes that the population sizes and migrations rate between pairs of populations were constant over the coalescence period (about $4N_e$ generations) (Austin et al. 2004). MIGRATE uses estimates of gene genealogies to sample areas of the coalescence space with highest likelihoods. MIGRATE was run according to the author's recommendations. The random number of seed and starting values of θ and $4N_em$ were based on F_{ST}, and the subsequent run used the θ and $4N_em$ from previous runs as starting parameters (Beerli 2006). A Brownian mutation model was assumed and the data were analysed using 10 short (10^4MCMC steps) and five long chains (10^5 MCMC steps), together with "adaptive heating" based on four temperatures, one "cold" and three "hot" chains to assure convergence.

To estimate the direction and rate of contemporary migration over the most recent generations and migrant ancestries, a Bayesian method based on multilocus genotypes implemented in BAYESASS was used (BAYESASS, Wilson & Rannala 2003). The method does not assume populations are at genetic equilibrium or that genotypes are in accord with Hardy-Weinberg equilibrium, but the loci in the parent populations are assumed to be in linkage equilibrium. The method is based on MCMC methods to estimate the posterior probabilities of the migration matrix among sub-populations (Wilson & Rannala 2003). Convergence was achieved after 6×10^6 MCMC iterations and a burn-in of 2×10^6 steps. The data were run three times, and the one where the log-likelihood had peaked and with the highest log-likelihood, was chosen.

Isolation by distance was estimated using geographical distances measured between the cores of the distribution areas of the populations in question using the shortest straight-line distance. The relationships between the genetic and geographical distances amongst the populations were estimated using a Mantel test implemented in ARLEQUIN v. 2.0 (Schneider et al. 2000).

Objective 3 - Historical and contemporary effective population size

The heavy exploitation by sealers during the 18th, 19th and early 20th centuries in some areas (Born et al. 1995) could have caused a significant reduction in genetic variation of walruses. Therefore, we tested for bottleneck effect in all sampling areas using the M-ratio (Garza & Williamson 2001) and BOTTLENECK 1.2 (Piry et al. 1999). The M-ratio contrasts the number of alleles, k, and the overall range in allele size, r; when population size declines, the number of alleles drops faster than the range, leading to smaller M values The parameters used in the present study were p_s (percent one-step mutations) = 80% (90%), Δ_g (mean size of non one-step mutations) = 3.5 and $\mu = 5x10^{-4}$ as recommended by Garza & Williamson (2001). Historical effective population size (θ) was estimated from MIGRATE (Beerli 2006, Beerli & Felsenstein 2001) (see above), while contemporary N_e was obtained from a linkage disequilibrium method (Waples 2006, Hill 1981, see below). In contrast to the M-ratio, BOTTLENECK 1.2 (Piry et al. 1999) is designed to detect an incompatibility between heterozygosity and the number of alleles per locus; following a decline in population size, the reduction in allele numbers occurs faster than the loss of heterozygosity, resulting in an apparent heterozygosity excess (Nei et al. 1975, Cornuet & Luikart 1996).

To evaluate the performance of BOTTLENECK we used EASYPOP v.1.7 (Balloux 2001) to simulate isolated populations segregating for 11 variable loci (as in our data) following a k-allele mutation model (each mutation equally likely to lead to any of k allelic states) and mutation rate μ= $5x10^{-4}$. Each replicate population ran for 5,000-10,000 generations to reach mutation-drift equilibrium before sampling. First, a single panmictic population with N_e=10,000 was simulated. After reaching mutation-drift equilibrium, the population was split into 50 sub-populations each having N_e=200 and tested in BOTTLENECK. The bottleneck size (N_e=200) was maintained for 50 generations, at which time 50 individuals were sampled from each population and tested in BOTTLENECK. This procedure was repeated with bottleneck size N_e=1,000 and with a constant N_e=10,000 (as a control; no bottleneck).

Under the assumption of the two-phase mutation model (TPM) (Di Rienzo et al. 1994) allowing for 90% single-step mutations and 10% multi-step mutations and a variance of 12%, the test for bottleneck was performed using the Wilcoxon test implemented in the program BOTTLENECK v. 1.2 (Piry et al. 1999). This test evaluates whether the number of loci with heterozygote excess is larger than expected to occur by chance alone; it is non-parametric, so is better suited for our analyses involving 11 loci than the standard deviate test also available under BOTTLENECK, which ideally requires 20 of more loci (G. Luikart, pers. com. September 2007).

The historical effective population size was estimated using the coalescence approach in MIGRATE (Beerli 2006) as described under *Objective 2*. Recent effective population size was estimated using the linkage disequilibrium method, which depends on random associations of alleles at different gene loci. The squared correlation of allele frequencies at pairs of loci ($\hat{r}^2$) was calculated using the composite Burrows method. This method, which is simpler than maximum-likelihood methods and does not require the assumption of random mating, is the preferred method for use with genotypic data such as are available for this study (Weir 1996). To estimate $\hat{N}_e$ from $\hat{r}^2$ we used the program LDNe (Waples & Do 2008), which implements the bias correction method described by Waples (2006). To minimize possible bias from low frequency alleles, we only used alleles with a frequency ≥0.02; this criterion appears to provide a good balance between maximizing precision and minimizing bias with highly polymorphic loci like microsatellites (Waples & Do 2008 and unpublished data). Confidence intervals to $\hat{N}_e$ were computed using Equation 12 in Waples (2006).

As the LDNe method can be biased by population mixture, we used STRUCTURE, BAYESASS and GENECLASS 2 (Piry et al. 2004) to identify migrants. Individuals identified as migrants by at least two of the assignment methods were moved to the identified area of origin before estimating the effective population size. Hence, the sample sizes in some areas decreased while it was increased in others (Table 1). In GENECLASS v. 2 (Piry et al. 2004) the likelihood of the individuals genotype was estimated using $L = L_{HOME}/L_{MAX}$ (Paetkau et al. 2004) and the Bayesian computation criteria of Rannala & Mountain (1997). Significance of the assignment of individuals at the 5% level was assessed based on the MCMC resampling option described by Paetkau et al. (2004). GENECLASS was only used to identify migrants for the replacement to other areas.

The sequential Bonferroni procedure was applied in table-wide tests of HWE, $F_{ST,}$ number of loci with significant different allele distribution and bottleneck at the 5% significance level (Rice 1989).

Results

After application of the sequential Bonferroni procedure (Rice 1989) no overall deviations from the HWE were observed in the Hudson Strait sample (Table 2). No linkage disequilibrium was observed between all pairs of loci within the different sampling areas (data not shown), hence the prior assumptions regarding linkage equilibrium in the parent populations behind the method implemented in STRUCTURE (Pritchard et al. 2000) are not violated.

Objective 1-Estimation of the number of sub-populations

All the F_{ST} estimates from the pair-wise comparisons (except Franz Josef Land -Svalbard) revealed statistically significant differences among the six sampling areas (Table 3). The pairwise F_{ST}-estimates over all loci ranged from 0.021 between the Hudson Strait and W Greenland samples to 0.069 between the Hudson Strait and NW Greenland samples (Table 3). The STRUCTURE analysis found by far the strongest support for the existence of five genetically distinct sub-populations: Hudson Strait, W Greenland, NW Greenland, E Greenland and Franz Josef Land -Svalbard (Ln probability of data Ln P (D): *k=4, ln* = -*9506.6; k=5, ln = -9327.8; k=6, ln = -9652.23*) (Fig. 2). Under the assumption that k = 5, the clusters identified by STRUCTURE largely corresponded to the 5 sampling locations identified as distinct in the F_{ST} analyses: (E Greenland, W Greenland, NW Greenland, Hudson Strait, and Franz Josef Land -Svalbard; Table 4 and Fig. 2). STRUCTURE was not able to identify a separate Franz Josef Land and Svalbard sub-population for higher numbers of clusters (k).

The low F_{ST} estimate between Hudson Strait and W Greenland suggests that these two groups of walruses might have diverged recently or are still exchanging migrants. A crude estimate of the time for divergence (t, in generations) between W Greenland and Hudson Strait based on the F_{ST} can be obtained from the expectation that $t \approx 2N_eF_{ST}$ (Hartl & Clark 1997).

Given the harmonic mean of the effective population size from the two populations in terms of θ ($4N_e\mu$, Fig. 3a), and using the 95% confidence intervals of θ and F_{ST} and an average microsatellite mutation rate of 5 x 10^{-4} (Weber & Wong 1993), the estimated divergence time started 4-18 generations ago. Assuming a generation time of about 15 years this corresponds to some time during the 1700s-1900s.

Objective 2 - Estimation of historical and contemporary migration patterns

The analyses of migration patterns west of Greenland indicated that estimate of the historical and recent migration direction was predominantly from Hudson Strait to W Greenland with a moderate average number of historical migrants per generation (Nm=2.48*)* (Fig. 3a). The equilibrium model (MIGRATE) suggested asymmetrical historical migration from NW Greenland to W Greenland, but this pattern was not reflected in the estimate of the recent direction, which indicated very little interchange between these two areas. Both historical and recent migration direction and average exchange of migrants per generation between Hudson Strait and NW Greenland were balanced and small (Fig. 3a,b).

For the sub-populations east of Greenland (Fig.3c), the historical migration direction went from E Greenland to Franz Josef Land -Svalbard. In contrast, the more recent estimates (Fig. 3d) reflected a symmetrical and low migration between the two areas.

Comparing migration directions between sub-populations west and east of Greenland, the historical migration direction went from NW Greenland to Franz Josef Land -Svalbard (Fig. 4a). Between NW Greenland and E Greenland (Fig. 4a) the historical migration was symmetrical and lower. However, the more recent migration direction and rate was low and symmetrical (Fig. 4b) between NW Greenland and E Greenland and Franz Josef Land - Svalbard. In W Greenland, both the historical and contemporary methods suggested a migration direction going from E Greenland to W Greenland, while the direction was symmetrical between W Greenland and Franz Josef Land -Svalbard (Fig. 4c,d). A moderate historical and symmetrical migration direction was estimated between Hudson Strait and Franz Josef Land –Svalbard (Fig. 4e,f), but this was not reflected in the more recent estimate, where the migration rate was low between these two sub-populations. Between Hudson Strait and E Greenland, both the historic and recent migration direction and rate were estimated to be symmetrical and low.

No significant correlation was observed between genetic and geographical distances among the walrus sub-populations based on a Mantel test implemented in ARLEQUIN (data not shown).

Objective 3 - Historical and recent estimates of effective population size

For the sub-populations west of Greenland, likelihood estimates of the historical effective population size in terms of θ decreased from 0.693 in Hudson Strait to 0.372 in NW Greenland and 0.327 in W Greenland (Fig. 3a). The same pattern of relative effective size was found in the estimates of contemporary N_e based on linkage disequilibrium (Fig. 3b).

The historical population sizes in the sub-populations east of Greenland were at comparable levels (Fig. 3c), while the contemporary N_e estimate was an order of magnitude lower for the E Greenland sample (N_e =98, 95%CI: 63-184) compared to the estimate for Franz Josef Land-Svalbard sample (N_e = 1264, 95% CI: 182-infinity) (Fig. 3d).

After application of the sequential Bonferroni correction no indication of a bottleneck was detected in any of the walrus sub-populations (data not shown). The results of the simulations to test the performance of BOTTLENECK 1.2 give some insight to the power to detect bottlenecks. In the control runs, none of the populations showed indications of a bottleneck (data not shown). Testing the 50 individuals from the populations with bottleneck N_e=200, 41 of the 100 showed a significant heterozygote excess using the Wilcoxon test.

Finally, only eight of the 100 populations with a bottleneck of N_e=1000, showed significant heterozygote excess, just slightly more than would be expected by chance.

Discussion

Objective 1-Estimation of the number of sub-populations

The genetic differences based on pairwise F_{ST} estimates (Table 3) suggested that five sub-populations of Atlantic walruses were present: Hudson Strait, W Greenland, NW Greenland, E Greenland and Franz Josef Land-Svalbard. The significant F_{ST} estimate observed between Hudson Strait and W Greenland sub-populations was small and comparable to the level observed between Franz Josef Land and Svalbard, although the latter failed to reach statistical significance and has previously been shown to constitute one sub-population (Wiig et al. 1996, Andersen et al. 1998, Born et al. 2001). Furthermore, the Bayesian clustering analysis implemented in STRUCTURE confirmed the separation into five different gene pools i.e. the analysis identified Hudson Strait and W Greenland as different gene pools and Franz Josef Land and Svalbard as belonging to the same gene pool also with increasing numbers of clusters (k). The identification of W Greenland, NW Greenland, E Greenland and Franz Josef Land-Svalbard sub-populations confirms previous studies (Andersen et al. 1998, Born et al. 2001), whereas the finding that walruses from Hudson Strait differ genetically from walruses in the other areas is novel (Table 4, Fig. 2).

Genetic structure at a comparable geographical scale has been observed in other pinniped species with an affinity to terrestrial haul-out sites (Allen et al. 1995, Stanley et al. 1996, Goodman 1998, Slade et al.1998, Wynen et al. 2000, Trujillo et al. 2004). These studies have been based on more traditional approaches, such as F_{ST} and AMOVA analyses. This finding suggests that at least those pinnipeds that rely on regular access to haul-outs on land, exhibit pronounced genetic population sub-structure. This is probably related to their dependence on such haul-out sites which may influence the social structure of the species.

The estimate of divergence time between Hudson Strait and W Greenland suggests that they might have diverged some time during the "Little Ice Age" (LIA). The LIA was a long, sustained cold period in the North Atlantic from 1300-1400 to 1900 (Grove 2001, Przybylak 2003). This period was characterized by particularly severe winters and an average temperature, slightly cooler than in present-day Greenland (Dahl-Jensen et al. 1998, Przybylak 2003). During the LIA, glaciers advanced in Greenland and the pack ice extended further south (Reiter 2000). In the late 1500s, Atlantic walruses lived (and probably reproduced) in areas farther south than at present: Sable Island (44° N) off Nova Scotia (Allen 1880, Allen 1930) and possibly the Orkney Islands in Scotland (59° N**)** (Ray 1960). The extended pack ice and the severe winters may have impacted the distribution of walruses during the mating period by restricting and separating suitable wintering locations. Born et al. (2001) proposed that the walruses in the three areas to the west of Greenland belonged to the same ancestral population. Their suggestion-- that the genetic divergence (based on both mitochondrial and microsatellite markers) between NW Greenland and W Greenland and walruses to the east of Greenland could be attributed to a separation caused by the existence of massive ice masses during the last glaciations period-- is supported by the results of the present study. However, intensive exploitation by commercial sealers and whalers during the 19th century (Born et al. 1995 and references therein) might have reduced the range of the species, adding to the geographical separation of the groups of walruses.

Objective 2 - Estimation of historical and contemporary migration patterns

West Greenland

It is not possible to compare the levels of historic and recent (contemporary) gene flow and dispersal rate directly. The gene flow is estimated in terms of effective number of migrants averaged over the coalescence time ($4N_e$ generations) and the dispersal rate is the proportions of migrants between the populations over the past 2-3 generations ago (Austin et al. 2004). However, assuming that the sub-populations were at mutation-drift equilibrium over the geographical range, patterns of migration direction can be evaluated. Given the hypothesised historical counter-clockwise migration movement pattern of walruses in the Baffin Bay region (Freuchen 1921, Vibe 1950), the direction of migration captured by the genetic signal is expected to go from the Hudson Strait to W Greenland and may be to NW Greenland. If this movement has been constant over a long period of time, we would expect the two different methods (i.e. MIGRATE and BAYESASS) to indicate similar patterns. Both historical and contemporary migration pattern implied that Hudson Strait functions as a source for W Greenland, hence supporting the counter-clockwise movement of walruses in the area. The discrepancy observed between the estimates of magnitude of gene flow using the two approaches may have been caused by the fact that samples of walruses from all areas in the Baffin Bay region were not included in this study (e.g. eastern Ellesmere and Baffin Islands; cf. Born et al. 1995). In addition, the sample sizes analysed might not be representative of the entire population (Beerli 2004) and not all assumptions behind the different methods applied (i.e. MIGRATE assumes a constant effective population size over several years) are met.

Apparently, walruses previously migrated from NW to W Greenland, but the more recent estimate (Fig. 3) suggests that this migration has ceased. Given the similar level of genetic differences between NW Greenland and W Greenland and between NW Greenland and Hudson Strait, the estimated divergence time for both pairs (1700-1900s) coincides roughly with the “Little Ice Age” as the event influencing genetic drift. We found no evidence that the migration pattern has changed over time between the NW Greenland and Hudson Strait.

The recently described performance problems (Faubet et al. 2007) observed in the algorithm used in BAYESASS (Wilson & Rannala 2003) were shown to be related to violation of the underlying demographic model, the level of genetic differentiation and the number of individuals and loci in the analysis. The present study largely met the lower bound of the critical criteria’s observed for the above mentioned suggested requirements i.e. the walrus populations exchange migrants not extremely different from the Wright island model, F_{ST} ~0.05, close to equality of the number of individuals, and the number of loci used (11) was close to the suggested 15 loci (Faubet et al. 2007). Based on the present study we believe that Hudson Strait, W Greenland and NW Greenland are genetically different sub-populations that are connected through migration and diverging slowly.

East Greenland

The change in migration pattern observed in E Greenland may be reflected in the crude estimate of the time for divergence. Given the level of differentiation (F_{ST}= 0.038, 95%CI 0.02-0.052), the time of divergence started around year 1400-1800s likewise suggesting LIA as the probable event driving the separation of the sub-populations to the east (i.e. Franz Josef Land -Svalbard).

Between west and east Greenland

The analysis of migration direction and rate between west and east Greenland during a longer time span supported the earlier hypothesis of a common ancestral walrus population in the North Atlantic about the time of the end of the last Ice Age 12,000 years ago (Born et al. 2001). This hypothesis is corroborated by the fossil finds of walruses on the Atlantic coast of North America, that indicate a northern limit of their Last Glacial Maximum (LGM) “refuge” near Long Island (New York, ca. 43° 57' N) that advanced to central Canada Arctic by 9,700 BP and to the Bay of Fundy (Nova Scotia, ca. 45° 0' N) by 7,500 BP (Dyke et al. 1999). In the North Sea and Denmark finds of 24,000–31,000-year-old walrus fossils indicate that walruses were a "Danish" faunal element during the last Ice Age (Møhl 1985). At that time a huge ice sheet covered Scandinavia (e.g. Hughes et al. 1981), and the walrus probably lived close to its northernmost border. However, in those areas of the North Atlantic where walruses lived during the LGM there was no vast continental shelf. This reduction in the range of suitable walrus habitat in the western and eastern Atlantic, and likely also in walrus abundance, probably caused a "bottleneck" effect that could explain the genetic differences between walruses to the west and to the east of Greenland (Andersen et al. 1998, Born et al. 2001). Furthermore, the dispersal between the populations would also be restricted as indicated by the recent migration/dispersal estimates in the present study, hence indicating that the more sedentary behaviour observed during the last century is reflected in the genetic make up.

In the Atlantic walruses, the patterns of "isolation by distance" depend on the geographical scale of interest. Andersen et al. (1998) did not detect "isolation by distance" among walruses in NW Greenland, E Greenland and Franz Josef Land-Svalbard. Including W Greenland in the "isolation by distance" analysis, Born et al. (2001) detected a significant isolation by distance pattern. In the present study, the Hudson Strait sample was included in the "isolation by distance" analysis together with the other four sub-populations. This inclusion increased the geographical distance between the sub-populations, which, together with the genetic divergence observed between areas, disrupted the significant "isolation by distance" pattern. This suggests that factors other than geographical distance may be just as important (for example the last Ice Age, 18,000 years ago) as described for the southern elephant seal (*Mirounga leonina*) by Slade et al. (1998).

Objective 3 - Historical and recent estimates of effective population size

The difference between historical and recent effective population size observed in the E Greenland sample could be a result of heavy commercial exploitation in the late 19th and early 20th centuries (cf Born et al. 1997). In the 1950s, walruses almost disappeared from eastern Greenland and Svalbard, when regulations to protect them were introduced (Gjertz & Wiig 1994, 1995; Born et al. 1997). However, the recent effective population size estimate might be biased downwards due to lack of proper identification of migrants from different sub-populations and low sample sizes, both factors that reduce the power of the LDNe method and generally led to wide confidence intervals for the point estimates.

No significant reduction in population size was detected in any of the sub-populations using both tests for bottlenecks. The M ratio test is regarded as a more informative test for the detections of more contemporary bottlenecks (between 125-500 generations or even more recent) (Garza & Williamson 2001) when (i) these have lasted for several generations, (ii) the population has recovered demographically and (iii) the pre-bottleneck population size was high or mutation rates high (Williamson-Natesan 2005). If the bottleneck was more recent and less severe and pre-bottleneck population size was low or mutation rate was low, the test for heterozygosity excess would be more likely to identify a bottleneck (Williamson -Natesan 2005).

The simulation tests based on the demography parameters for the Atlantic walrus and the observed genetic diversity at the 11 loci indicated that BOTTLENECK 1.2 (Piry et al. 1999) could detect a significant reduction in population size after 50 generations when the bottleneck size was N_e=100 or N_e=200, but not when N_e=1000. However, simulation results show that with bottleneck $N_e = 200$, the power of the algorithm used in BOTTLENECK 1.2 is less than 50% for datasets comparable to those for the walrus. The bottleneck in the walrus sub-populations east of Greenland occurred in the 18th and 19th centuries (~28 walrus generations) so one explanation could be that the reduction in size happened too recently to be detected or N_e has not been as low as suggested by the catch statistics (Born et al. 1997, Gjertz et al. 1998). According to this hypothesis, the walruses were not extirpated, but moved somewhere else or survived in more remote and less accessible areas (e.g. the northernmost parts of NE Greenland and Franz Josef Land). Another explanation could be that the underlying assumption of the mutation model does not fit the microsatellite loci used.

Management implications for the walruses west of Greenland

This study indicates that the role of Hudson Strait walruses as a source for walruses hunted on the wintering grounds in W Greenland has been constant over several centuries. The more sedentary behaviour suggested by Mansfield (1973) may not yet have resulted in a cease in the movements of some walruses from Hudson Strait to W Greenland. Furthermore, the results indicate that during the LIA the distribution of the sea ice probably restricted the movement of walruses and influenced their access to various foraging grounds so that the populations started to diverge into more isolated sub-populations.

This information is important for the management of the walruses. Differences in the genetic make-up of walruses from different geographical areas studied indicate that they represent sub-populations and should be managed separately. However, the study also indicated the existence of some contemporary migration between the putative sub-populations in the Baffin Bay area – a fact that must be considered when setting sustainable catch levels for the various areas. The indication that such connections have existed for several centuries might explain why the genetic variation observed in the W Greenland sample was at the same level as observed in the samples from Hudson Strait and NW Greenland despite estimates of abundance in west Greenland only being in the low thousands (Born & Witting 2005, NAMMCO 2006, Heide-Jørgensen et al. 2006).

Walruses are completely protected in Svalbard and Franz Josef Land (cf. Gjertz & Wiig 1994, Born et al. 1995) and are thought to be taken at sustainable level in E Greenland (NAMMCO 2006). However, exploitation is believed to be the main limiting factor and threat to the Atlantic walrus in certain parts of Canada including the stocks shared with NW Greenland and W Greenland (NAMMCO 2006, COSEWIC 2006, Stewart 2008). Walruses are still hunted for subsistence purposes in E Greenland, W Greenland, NW Greenland and the Hudson Strait (NAMMCO 2006, COSEWIC 2006, Stewart 2008). Until 2006 the catch of walruses in Greenland was not regulated by any quota system, whereas the catch of walruses in Canada has been regulated for decades by either individual bag limits or settlement/community quotas (COSEWIC 2006).

Although estimates of abundance in several of the areas occupied by sub-populations are inadequate, the estimates and qualified guesstimates indicate that the different sub-populations of Atlantic walruses number only few hundreds to few thousands (Born et al. 1995, NAMMCO 2006, COSEWIC 2006). Given the uncertainty connected with the estimates of the effective population size (N_e) in the present study and the lack of population estimates for most of the sub-populations, the N_e/N ratio was not estimated. However, this relationship could be a tool for management in order to follow the importance of the removals on the survival of the sub-populations.

Walruses occur along the SE coast of Baffin Island (Born et al. 1995, Stewart 2008) vis-à-vis the W Greenland walrus grounds. Walruses on SE Baffin Island may well be connected to the W Greenland walruses as suggested by Born et al. (1994). The existence of this connection has been supported by recent satellite telemetry where walruses instrumented in W Greenland moved to SE Baffin Island during spring (Greenland Institute of Natural Resources and Danish National Environmental Research Institute unpublished data). Hence, walruses exploited in W Greenland can be winter migrants from a sub-population that also occurs in SE Baffin.

Currently, the management of the shared populations of Atlantic walruses in Canada and Greenland is not formally coordinated. However, the fact that the W Greenland and NW Greenland sub-populations are shared calls for a coordinated management. An important part of such management is identification of sub-populations or management units. The present study is a contribution to identification of management units. However, efforts to determine the identity of sub-populations shared by Canada and Greenland continue.

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Figure 1

Map showing the approximate distribution of Atlantic walrus sub-populations (after Born et al. 2001). Samples included in the present study came from areas inhabited by the putative stocks numbered 2, 3, 4, 5, 6 and 7. Red circles indicate sampling sites and blue arrows show the described counter-clockwise migration movements of walruses west of Greenland.

Figure 2

Graphical output from STRUCTURE (Pritchard et al. 2000) for *k* = 5. Each vertical line represents an individual, and the colour composition displays the probability of belonging (proportion of membership) to each of the five clusters defined by STRUCTURE.

Figure 3

Estimates (a) of the effective historical population size (θ) gene flow and migration directions (numbers and arrows) for the populations west of Greenland estimated in MIGRATE (Beerli 2006), b) estimate of recent effective population size (N_e) using linkage disequilibrium (Waples 2006) and migration rate and directions for the populations west of Greenland estimated in BAYESASS (Wilson & Rannala 2003), c) results of the effective historical population size (θ) gene flow and migration directions (numbers and arrows) for the populations east of Greenland estimated, d) estimate of recent effective population size (N_e) and migration rate and directions for the populations east of Greenland (WGR= W Greenland, NWGR= NW Greenland, HS= Hudson Strait, EGR= E Greenland, FJL-SVA= Franz Josef Land-Svalbard). Numbers in brackets () = 95%CI. Numbers in italics indicates proportions of migrants where data are considered to be informative. Means of 0.833 (0.675-0.992 (95%CI)) for non-migrants and 0.032 (1.58E-06-0.160 (95%CI)) for migrants observed in the analysis of the six sampling locations indicated that the data did not contain sufficient information.

Figure 4

a) Results of the historical gene flow and migration directions (numbers and arrows) for the populations between NW Greenland and E Greenland, Franz Josef Land-Svalbard estimated in MIGRATE (Beerli 2006), b) recent migration rate and directions estimated in BAYESASS (Wilson & Rannala 2003), c) historical gene flow and migration directions between W Greenland (WGR) and E Greenland (EGR), Franz Josef Land – Svalbard (FJL-SVA), d) estimate of recent migration rate and directions, e) historical gene flow and migration directions between Hudson Strait and E Greenland (EGR), Franz Josef Land-Svalbard (FJL-SVA), f) recent migration rate and directions. Numbers in brackets () = 95%CI. Numbers in italics indicates proportions of migrants where data are considered to be informative. Means of 0.833 (0.675-0.992 (95%CI)) for non-migrants and 0.032 (1.58E-06-0.160 (95%CI)) for migrants observed in the analysis of the six sampling locations indicated that the data did not contain sufficient information.

Table 1. Summary of samples used for genetic analysis from eastern Canada, Greenland and Franz Josef Land and Svalbard. Putative stock numbers from Born et al. (1995) and Figure 1.

Region	Putative Stock	N	F	M	N**[d]	Abbreviation in text	Sampling year
Foxe Basin[a]	1	-				-	
Northwest Greenland	5	72	29	43	59	NWGR	[b]1990, 1991
West Greenland	4	33	18[c]	11[c]	16	WGR	[b]1988, 1989, 1997
Hudson Strait	3	58	16	42	64	HS	new material 1998-2000
East Greenland	6	76		76	74	EGR	[b] 1989, 1990 new material 1999-2000
Svalbard	7	28		28	56[e]	SVA	[b]1992
Franz Josef Land	8	30	16	14	56[e]	FJL	[b]1992
Total		297	79	214			

[a] Foxe Basin sub-population recognized by Born et al. (1995) but not included in the present study

Born et al. 2001

[b]) Samples used in Andersen et al. 1998

[c] 4 individuals were not sexed

[d] Sample sizes after identification of migrants and replacing those individuals to the identified origin (see text)

[e] Svalbard and Franz Josef Land were pooled as they probably belong to the same sub-population.

Table 2. Expected (H_e) heterozygosity, allele richness (AR), and goodness of fit to the Hardy-Weinberg expectations (F_{IS}) testing for heterozygosity deficiency in GENEPOP (Raymond & Rousset 1995) at the eleven microsatellite loci (Andersen et al. 1998) for the walrus samples from the six different areas.

	H_e	SD	AR	SD	F_{IS}
NW Greenland	0.647	0.056	6.55	2.63	0.055*
W Greenland	0.608	0.093	6.06	3.07	0.058
Hudson Strait	0.614	0.066	5.95	3.01	0.032
E Greenland	0.59	0.079	5.02	1.98	0.021
Svalbard	0.658	0.078	6.18	3.54	0.118**
Franz Josef Land	0.634	0.09	6.05	2.78	0.088

* Sig. deviation from HWE at Orr11

** Sig. deviation from HWE at Orr16 and overall

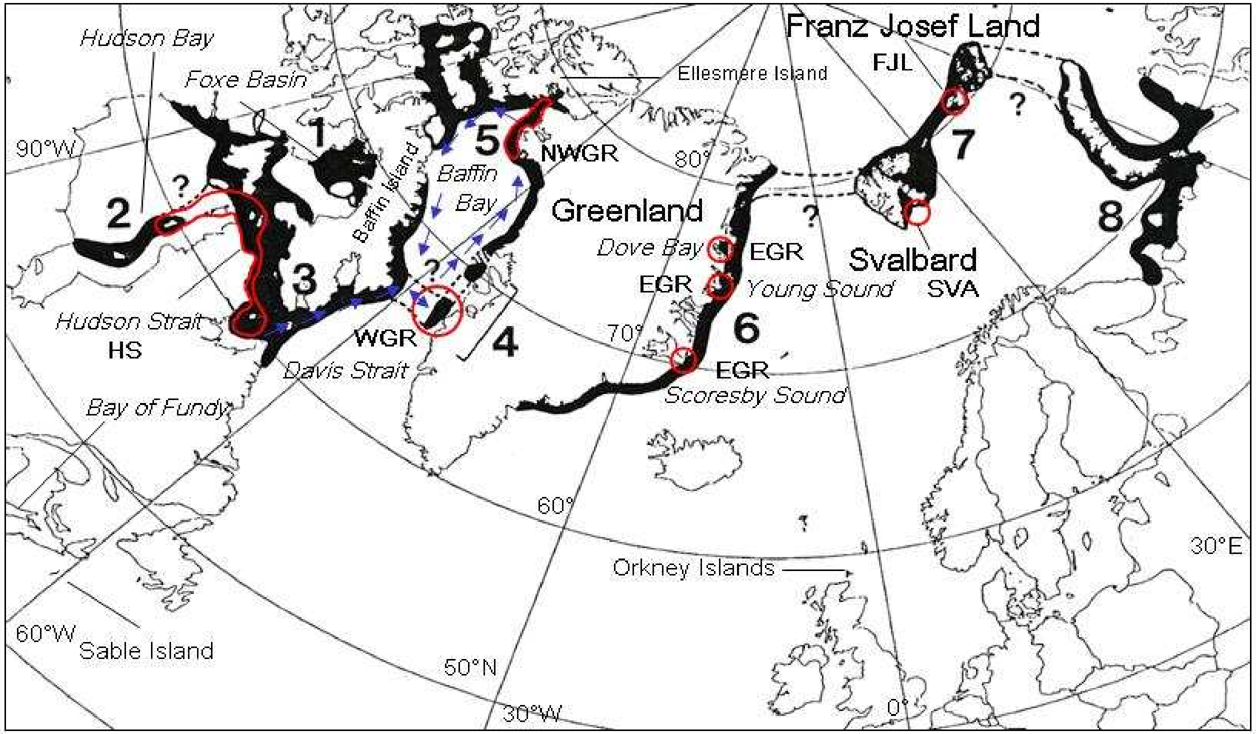
Table 3. Results of the pair-wise multilocus F_{ST} tests and the 95% confidence intervals for genetic differences between the five putative sub-populations of Atlantic walrus from Hudson Strait region, NW Greenland, W Greenland, E Greenland and the Svalbard-Franz Josef Land area. All tests performed in FSTAT after 10000 permutations (Goudet 1995). Bold= significant.

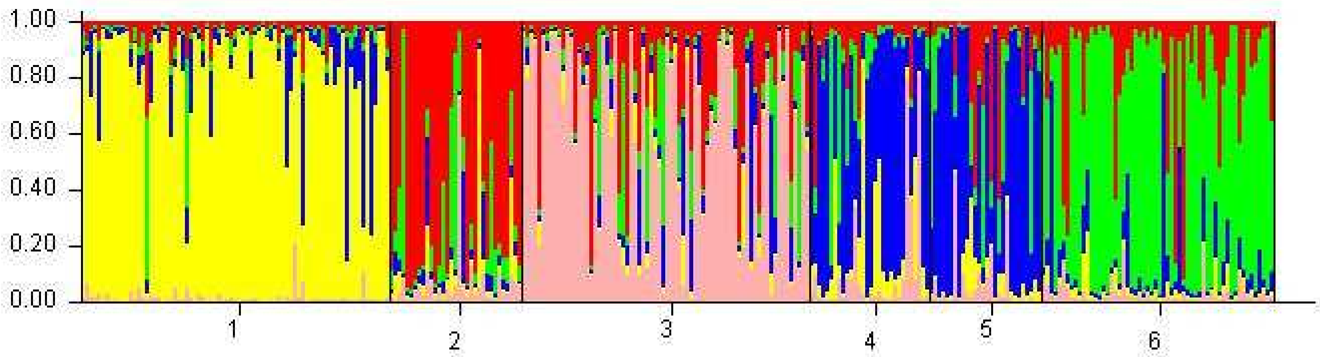
	NWGR	WGR	HS	EGR	SVA
West Greenland (WGR)	**0.035**				
95% CI	**(0.021-0.052)**				
Hudson Strait (HS)	**0.043**	**0.021**			
95% CI	**(0.025-0.058)**	**(0.009-0.036)**			
East Greenland (EGR)	**0.069**	**0.048**	**0.066**		
95%CI	**(0.039-0.112)**	**(0.027-0.068)**	**(0.044-0.091)**		
Svalbard (SVA)	**0.060**	**0.041**	**0.037**	**0.054**	
95%CI	**(0.037-0.086)**	**(0.019-0.068)**	**(0.019-0.060)**	**(0.048-0.094)**	
Franz Josef Land (FJL)	**0.046**	**0.027**	**0.034**	**0.037**	0.024
95%CI	**(0.030-0.065)**	**(0.012-0.042)**	**(0.021-0.050)**	**(0.020-0.054)**	(-0.005-0.063)

Table 4. Mean estimated proportion of membership (q) of the individuals into one of the five identified clusters in Fig. 2. (STRUCTURE, Pritchard et al. 2000). (NWGR= NW Greenland, HS= Hudson Strait, FJL-SVA= Franz Josef Land-Svalbard, EGR= E Greenland, WGR= W Greenland)

	Clusters suggested by STRUCTURE					
Sampling areas	NWGR	HS	FJL-SVA	EGR	WGR	N
NW Greenland	**0.611**	0.097	0.069	0.035	0.188	72
W Greenland	0.068	0.155	0.056	0.068	**0.654**	33
Hudson Strait	0.033	**0.675**	0.09	0.028	0.174	58
E Greenland	0.020	0.035	0.098	**0.807**	0.040	76
Svalbard	0.061	0.141	**0.610**	0.051	0.136	28
Franz Josef Land	0.109	0.105	**0.568**	0.132	0.087	30

30°


Hudson Bay
Foxe Basin
Franz Josef Land
FJL
Ellesmere Island
90°W
1
5
NWGR
Baffin Island
Baffin Bay
80°
2
?
7
?
8
Greenland
?
3
Dove Bay
EGR
Svalbard
SVA
EGR
Young Sound
Hudson Strait
HS
WGR
4
70°
6
Davis Strait
EGR
Scoresby Sound
Bay of Fundy
60°
30°E
Orkney Islands
60°W
Sable Island
50°N
30°W
0°



E Greenland W Greenland NW Greenland FJL Svalbard Hudson Strait

Figure 2

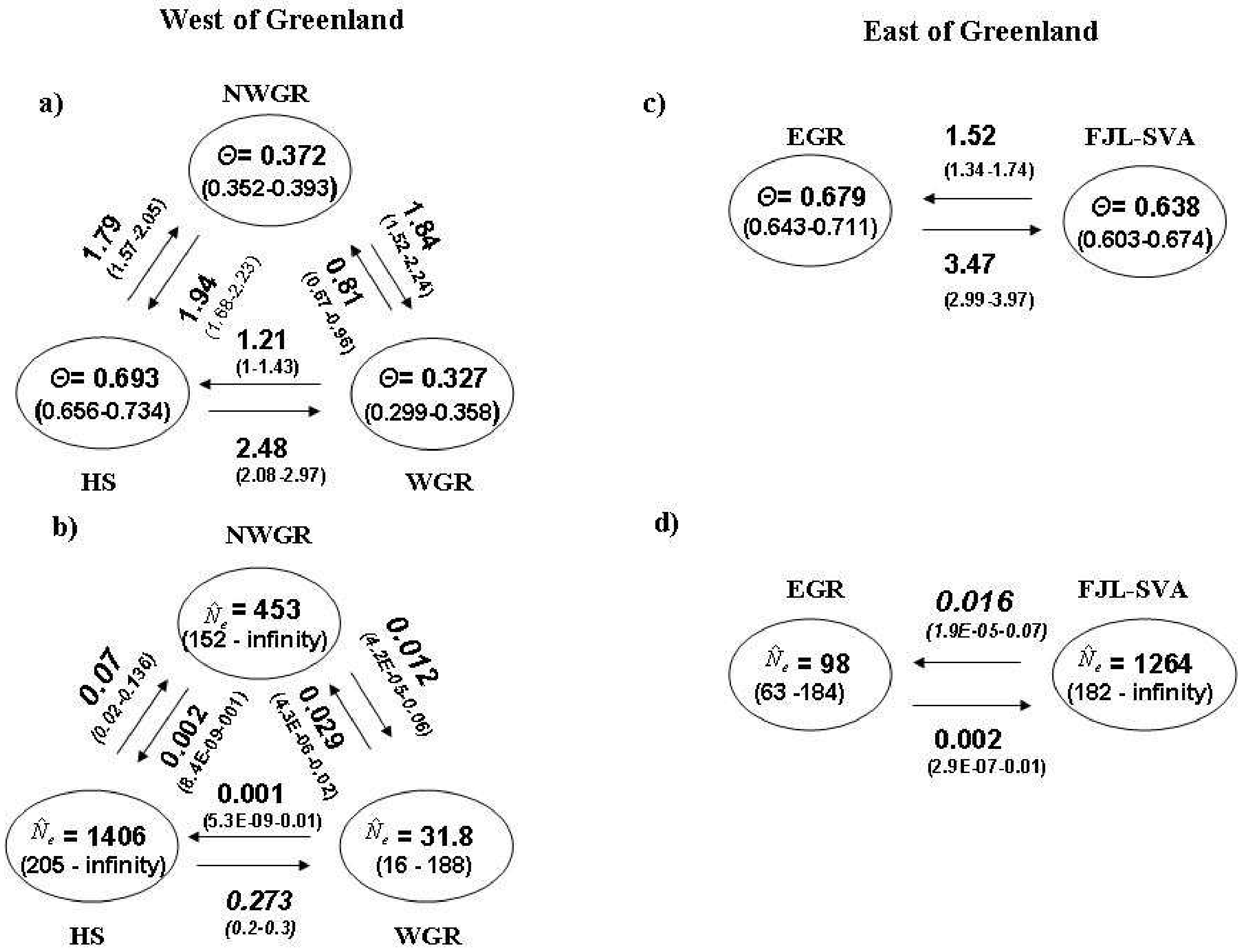

West of Greenland
East of Greenland
a)
NWGR
Θ= 0.372
(0.352-0.393)
1.79
(1.57-2.05)
1.94
(1.68-2.23)
1.84
(1.52-2.24)
0.81
(0.67-0.96)
1.21
(1-1.43)
Θ= 0.693
(0.656-0.734)
Θ= 0.327
(0.299-0.358)
2.48
(2.08-2.97)
HS
WGR
b)
NWGR
$\hat{N}_e$ = 453
(152 - infinity)
0.07
(0.02-0.136)
0.002
(8.4E-09-001)
0.012
(4.2E-05-0.06)
0.029
(4.3E-06-0.02)
0.001
(5.3E-09-0.01)
$\hat{N}_e$ = 1406
(205 - infinity)
$\hat{N}_e$ = 31.8
(16 - 188)
0.273
(0.2-0.3)
HS
WGR
c)
EGR
FJL-SVA
1.52
(1.34-1.74)
Θ= 0.679
(0.643-0.711)
Θ= 0.638
(0.603-0.674)
3.47
(2.99-3.97)
d)
EGR
FJL-SVA
0.016
(1.9E-05-0.07)
$\hat{N}_e$ = 98
(63 -184)
$\hat{N}_e$ = 1264
(182 - infinity)
0.002
(2.9E-07-0.01)

Figure 3

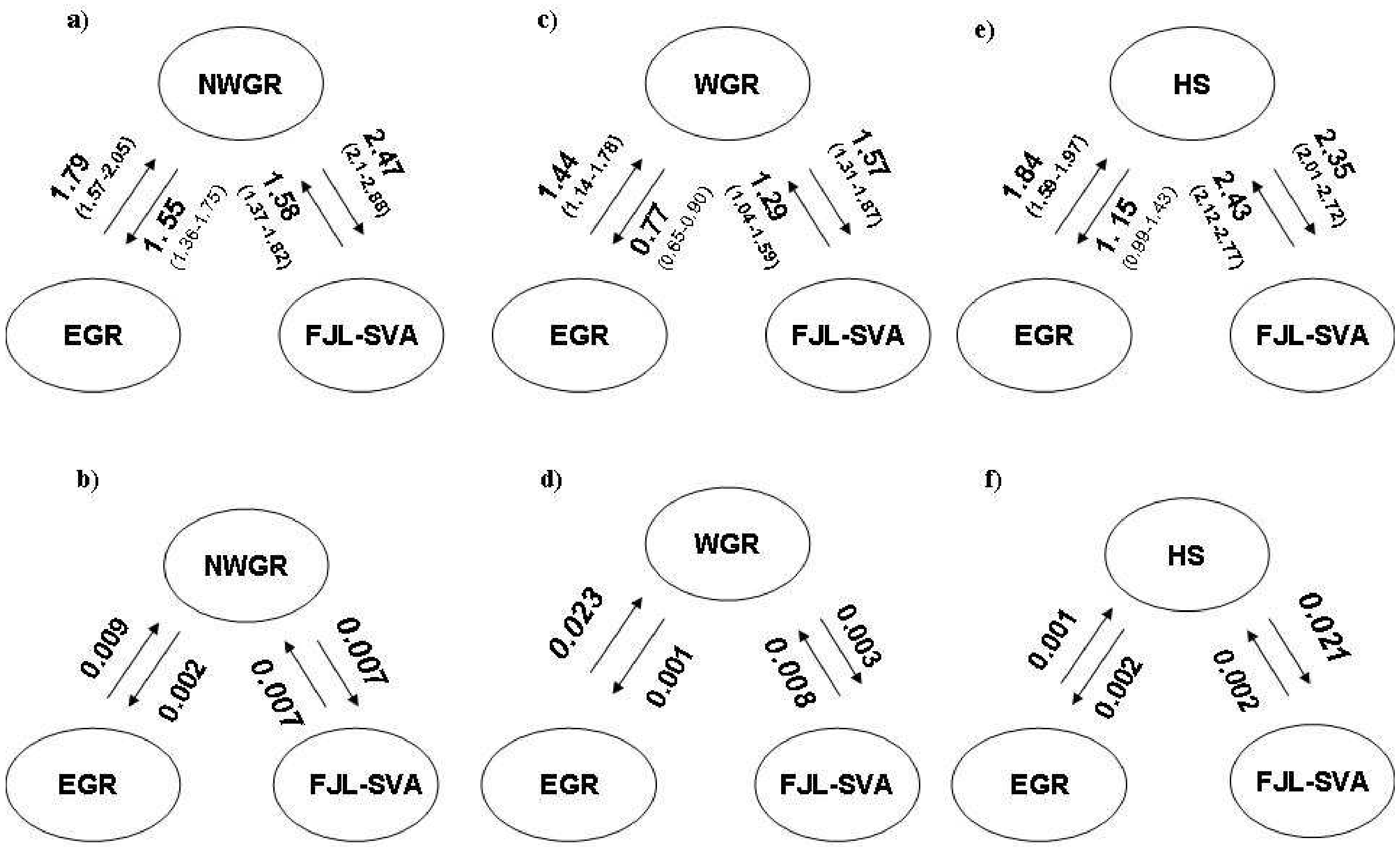

a)
NWGR
1.79
(1.57-2.05)
1. 55
(1.36-1.75)
1.58
(1.37-1.82)
2.47
(2.1-2.86)
EGR
FJL-SVA
c)
WGR
1.44
(1.14-1.78)
0.77
(0.65-0.90)
1.29
(1.04-1.59)
1.57
(1.31-1.87)
EGR
FJL-SVA
e)
HS
1.84
(1.59-1.97)
1. 15
(0.89-1.43)
2.43
(2.12-2.77)
2.35
(2.01-2.72)
EGR
FJL-SVA
b)
NWGR
0.009
0.002
0.007
0.007
EGR
FJL-SVA
d)
WGR
0.023
0.001
0.008
0.003
EGR
FJL-SVA
f)
HS
0.001
0.002
0.002
0.021
EGR
FJL-SVA

Figure 4