Fisheries and Oceans Canada | Pêches et Océans Canada
Science | Sciences

C S A S | *S C C S*

Canadian Science Advisory Secretariat | **Secrétariat canadien de consultation scientifique**

Research Document 2002/092 | **Document de recherche 2002/092**

Genetic relationships among Atlantic walrus (*Odobenus rosmarus rosmarus*) in the Foxe Basin and the Resolute Bay-Bathurst Island area.

Relations génétiques entre le morse atlantique (*Odobenus rosmarus rosmarus*) du bassin de Foxe et de la région Resolute Bay-Bathurst Island.

Brigitte G.E. de March, Lianne D. Maiers, and Robert E.A. Stewart

Fisheries and Oceans Canada,
501 University Crescent,
Winnipeg, MB, Canada R3T 2N6

ISSN 1499-3848 (Printed)

Canada

ABSTRACT

Maternally inherited mitochondrial DNA (mtDNA) and alleles at 13 nuclear DNA microsatellite loci were analysed for walrus from the putative Foxe Basin stock (Hall Beach n=71, Igloolik n=75 and from the putative Baffin Bay stock (Grise Fiord n=5 and Resolute Bay/Bathurst Island n=18). The data were examined for genetic differences among the 4 groups and also for annual differences within the 4 groups. Results strongly supported the hypothesis that Foxe Basin walrus and Baffin Bay walrus were different stocks, but did not support the hypotheses of further stock division. Hall Beach and Igloolik walrus were not significantly differentiated from each other, nor were Grise Fiord and Resolute Bay/Bathurst Island walrus. A lower than expected degree of heterozygosity within individuals indicated that some walrus were inbred. This is believed to be due to the harem mating system of walrus. Nevertheless, there is still a high degree of genetic diversity in walrus. The probability of sampling pairs of individuals that have a parent-offspring-like genetic relationship is quite high, approximately 0.01 for microsatellites and 0.001 for microsatellites and haplotypes combined. Therefore, it is difficult to identify true parent-offspring pairs in a large population. The use of more genetic markers would make this type of identification more certain. The use of molecular genetics to study the mating systems and movements of walrus is discussed.

RÉSUMÉ

On a analysé l'ADN mitochondrial (ADNmt), d'origine maternelle, et les allèles à 13 loci microsatellitaires d'ADN nucléaire de morses issus du stock putatif du bassin de Foxe (Hall Beach, n = 71; Igloolik, n = 75) et du stock putatif de la baie de Baffin (Grise Fiord, n = 5; Resolute Bay-Bathurst Island, n = 18). À partir des données obtenues, on a tenté d'établir s'il existait des différences génétiques entre les quatre groupes et des différences annuelles au sein d'eux. Les résultats appuient énergiquement l'hypothèse que le morse du bassin de Foxe et le morse de la baie de Baffin sont deux stocks différents, mais n'appuyait pas les hypothèses qu'ils pouvaient être subdivisés. Le morse de Hall Beach et le morse d'Igloolik n'étaient pas très différents l'un de l'autre, tout comme le morse de Grise Fiord et le morse de Resolute Bay-Bathurst Island. Un niveau d'hétérozygosité des individus plus faible que prévu indique que certains morses étaient consanguins. On croit que cela est imputable au type d'accouplement en harem de l'espèce, qui montre malgré cela un niveau élevé de diversité génétique. La probabilité d'échantillonner deux individus ayant une relation génétique du genre parent-descendant est assez élevée, se situant presque à 0,01 dans le cas des microsatellites et à 0,001 dans le cas des microsatellites et des haplotypes combinés. Il est par conséquent difficile d'identifier de vraies paires parent-descendant dans une grande population. Pour ce faire, il faudrait utiliser un plus grand nombre de marqueurs génétiques. Est aussi examinée l'application de la génétique moléculaire à l'étude des types d'accouplement et des déplacements du morse.

Introduction

At least four walrus stocks are assumed to occur in the Canadian eastern high Arctic (R.E.A. Stewart, paper this meeting, Born *et al.* 1995). These are the east and south Hudson Bay stock, the Hudson Bay-Davis Strait stock, the Foxe Basin stock, and the Baffin Bay stock. Walrus from Foxe Basin were thought to be part of one stock and the hunt of walrus is managed on the basis of this assumption (Cosens *et al.* 1993). Molecular genetics techniques allow us to test these stock hypotheses and to look for possible stock sub-divisions.

In this study, we examined genetic differences among 71 walrus from Hall Beach, 75 from Igloolik, both samples from the putative Foxe Basin stock, and 5 from Grise Fiord and 18 from Resolute Bay/Bathurst Island, both samples from the putative Baffin Bay stock.

Materials and Methods

Walrus samples were selected from available tissues collected by R.E.A. Stewart (Table 1). Samples were mostly from hunter killed walrus and a few from Bathurst Island were from biopsies from tagged walrus.

Nine microsatellite loci (OrrFCB2, OrrFCB3, OrrFCB7, OrrFCB9, OrrFCB11, OrrFCB16, OrrFCB21, OrrFCB23, and OrrFCB24) and methods used to analyse for them are described in Buchanan *et al.* (1998). The other loci (hg4.2, hg8.1, hg6.1 and pv9) are described in Allen *et al.* (1996).

A portion of the mitochondrial DNA (mtDNA) d-loop was amplified and sequenced using primers developed for harbour seals *(Phoca vitulina)* (Stanley *et al.* 1996). Direct sequencing was performed using an ABI Prism 377 automated sequencer with dRhodamine dye terminator chemistry (PE Applied Biosystems) and the resulting sequences aligned with MacVector ver. 3.5 (IBI). Variable positions were identified in a 266 base pair region of the aligned sequences and haplotypes assigned to each unique pattern.

Differences among sample groups were described with AMOVA or "Analysis of Molecular Variance" (Excoffier *et al.* 1992, Weir 1996) available in the "Arlequin" statistical package (Schneider *et al.* 1997). AMOVA is a linear modeling method designed for genetic data that produces estimates of variance components and combinations of these, such as F-type statistics which are analogs of genetic distance measures (Weir and Cockerham 1984, Michalakis and Excoffier 1996). Genetic distances between individuals were calculated as 0 or 1 for haplotypes, and as 0, 1, 2, or 4 differences at each microsatellite locus ("number of different alleles" choice in Distance Matrix Options in Arlequin). The significance of F_{st} values was estimated by using 10,000 permutations of the difference matrix.

A nested AMOVA design was used in view of the main hypotheses tested, that is, years were nested within locations, and locations were nested within 2 putative stocks. Because this analysis showed only differences between the two putative stocks, AMOVA was then performed within each putative stock. By doing this, the variance of groups tested within the Foxe Basin

groups is applied only to Foxe Basin comparisons, and the same applies to Resolute Bay/Bathurst Island comparisons.

Genetic relationships among 13 sample collections (Table 1) were also described with a phylogenetic tree. Cavalli-Sforza's "chord distance" between populations was used as a measure of genetic distance (Cavalli-Sforza and Edwards 1967), and the neighbour-joining method of Saitou and Nei (1987) was used to construct phylogenetic trees. Both microsatellite allele frequencies and haplotype frequencies were used to calculate chord distance. Chord distance was calculated to 6 significant digits using a Visual Basic program written by the first author. The Neighbour-Joining program in the PHYLIP statistical package (Felsenstein 1993) was used to construct the trees. The distance between the ends of the branches represent the degree of differences between the sample populations while the angles and the directions of the branching are arbitrary.

Expected genetic diversity or heterozygosity was calculated as

$$H_e = 1 - \Sigma_i(p_{li})^2$$

for each microsatellite locus, and as a mean,

$$\text{Mean } H_e = 1 - \Sigma_l \Sigma_i(p_{li})^2/m$$

for all microsatellite loci, where p_{li} is the frequency of the *i*th allele at the *l*-th locus, and *m* is the number of loci (p. 150, Weir 1996). The expected diversity or heterozygosity is the fraction of loci expected to be heterozygous if the population is at Hardy-Weinberg (HW) equilibrium, that is, if individuals are breeding randomly (Hardy 1908, Weinberg 1908). HW equilibrium of microsatellites alleles was tested in the 13 minor and 4 major sample populations (Table 1) using the "exact HW test" of Haldane (1954) available in GENEPOP software (Raymond and Rousset 1995), which uses the Markov chain algorithm of Guo and Thompson (1992). Also, F_{is}, an inbreeding coefficient, was calculated for loci as

$$F_{is} = 2\Sigma_i(T_{ii}/N_i)/(k\text{-}1),$$

where k is the number of alleles, T_{ii} is a measure of the excess of the i-th homozygote, N_i the number of observations of the i-th alleles in the sample, and N the total of all alleles at each locus (Robertson and Hill 1984). F_{is} is positive if individuals are less heterozygous than expected. F_{is} was calculated using GENEPOP software (Raymond and Rousset 1995).

Observed heterozygosity (H_o) was calculated as the frequency of heterozygotes at all 15 loci (p. 141, Weir 1996). This measure was calculated for individuals as well as for loci within populations. If many individuals have fewer heterozygous loci than expected, they might be inbred. If they have more heterozygous loci than expected, there may be factors promoting outbreeding. This measure is not correlated with expected or observed genetic diversity in a population.

The probability that two randomly sampled individuals have the same genotypes, or the "probability of identity", was calculated as

$$ID_e = \Sigma_i(p_{li})^4 + \Sigma_i\Sigma_{j>i}(2*p_{li}p_{lj})^2$$

for each microsatellite locus and as a product of the ID_e s for all loci (Paetkau and Strobeck 1994). The probability of identity was also calculated for haplotypes as

$$ID_{eh} = \Sigma_i(p_{li})^2 ,$$

which can also be used in the product to calculate an overall probability of identity for females and offspring.

Graphs of distributions of observed and expected **homozygosities** (not heterozygosities), indicating degree of relatedness in individuals, and of observed and expected genetic relatedness among pairs individuals based on microsatellites, are presented to complement the above analyses. Corrections for missing alleles were made when calculating the distribution of observed similarities by substituting the most likely probability of identity for that locus within a population. Expected distributions of similarity frequencies were generated with simulations from population allele frequencies.

All individuals were screened for possible "parent-offspring-like relationships" (POLRs), and observed and expected frequencies of POLRs were compared. Two individuals have a POLR if they have at least one allele in common at each locus. A POLR is an identifiable relationship, whereas other relationships such as sibling relationships, are not. Expected frequencies of POLRs were calculated using probability theory in a Visual Basic program written by the first author.

Results

Sample Differentiation

There was significant differentiation among walrus sampled the 4 major locations (Table 1) on the basis of microsatellites ($p = 0.0033$) and weakly on the basis of haplotypes ($p = 0.0900$) ("Among Groups", Tables 2 and 3). Partitioning of the degrees of freedom and sums of squares showed that Hall Beach and Igloolik samples were not significantly differentiated ($p = 0.8563$ for microsatellites, $p = 0.1202$ for haplotypes), Grise Fiord and Resolute Bay/Bathurst Island samples were not significantly differentiated ($p = 0.4985$ for microsatellites, $p = 0.7458$ for haplotypes), but the Foxe Basin walrus were significantly differentiated from the Baffin Bay walrus ($p = 0.0010$ for microsatellites, $p = 0.0088$ for haplotypes). F_{st} values (genetic distances) among the 4 major locations are shown in Table 4.

There was little differentiation among years in these 4 populations (overall test, $p = 0.9777$ for microsatellites and $p = 0.1996$ for haplotypes, Tables 2 and 3). However, haplotype frequencies differed among years for Hall Beach samples ($p = 0.0323$). Within-group AMOVA showed that this was due to Hall Beach 1993 differing from both 1992 ($p = 0.0027$) and 1996 ($p = 0.0193$). Examination of haplotype frequencies shows that this is due to several haplotypes, all at low frequencies, differing between the two years.

The phylogenetic tree generally reflects these differences (Figure 1). All Foxe Basin samples are grouped with each other, as are the Baffin Bay samples. The Baffin Bay samples may be more different from each other than the Foxe basin samples are.

Diversity

Microsatellite diversity or expected heterozygosity was similar in the 4 sample locations: 0.55 for Grise Fiord, 0.65 for Resolute Bay /BI and 0.66 for each of Hall Beach and Igloolik (Table 5). Foxe Basin samples had 44 haplotypes/127 individuals while Baffin Bay Samples had 6/12 (Table 6), for a total of 48 haplotypes/139 individuals.

The probability of two individuals having the same microsatellite genotypes ranged from 3.1×10^{-10} for Grise Fiord walrus to 3.0×10^{-14} for Igloolik walrus. Probabilities of identity for individual loci ranged from 0.798 for Locus Orrfcb3 for Resolute/Bathurst walrus to 0.010 for Orrfcb23 for Igloolik walrus (Table 5). The probabilities of identity for the two putative stocks are 2.398×10^{-14} for Foxe Basin walrus and 1.907×10^{-13} for Baffin Bay walrus (Table 6). The probability of sampling identical haplotypes in two animals from two different stocks is 0.0513 for Foxe Basin and 0.222 for Baffin Bay, thus overall probabilities of identity for the two stocks would be 1.23×10^{-15} and 4.234×10^{-14} when this statistic is appropriate.

Heterozygosity and disequilibrium

HW equilibrium was observed in only 9 of 52 locus x location tests (HW equilibrium marked by *s in Table 5). Inbreeding coefficients (F_{is}) were positive for most loci in samples from Hall Beach, Igloolik, and Resolute/Bathurst (Table 5). In tests for HW equilibrium in 13 location x year walrus collections, using all 13 loci combined, only collections with small sample sizes were at HW equilibrium, namely Hall Beach 1988 (n=3), Grise Fiord 1996 (n=5), Resolute 1992 (n=5), and Resolute 1993 (n=9).

In the Foxe Basin sample, more walrus than expected were homozygous at 6/13 and 7/13 loci, and fewer than expected were homozygous at 4/13 and 5/13 loci (Figure 2). 23/136 individuals fell above expected values, and 23/136 fell below expected values. A similar pattern occurs in the Baffin Bay sample, in which 4/22 individuals fell above expected values and 4/22 below.

The observed and expected patterns of relatedness differ for Foxe Basin walrus and for Baffin Bay walrus, but not for Baffin Bay *vs* Foxe Basin walrus (Figure 3). Within both Foxe Basin and Baffin Bay populations, there are more individual pairs than expected that have a small fraction of alleles in common, and fewer individual pairs than expected have an average number of alleles in common.

Parent-Offspring-like Relationships (POLRs)

POLRs existed in 48/9180 pairwise comparisons among Foxe Basin individuals; in 2/231 comparisons among Baffin Bay individuals; and in 4/2992 comparisons of Foxe Basin and Baffin Bay individuals. In the Foxe Basin stock, the probability of two random individuals having a POLR was 0.006072; in the Baffin Bay stock, 0.014115; and across stocks, it was 0.014115 (Table 6). Thus expected numbers of individuals with POLRs with random breeding and random sampling are 56, 3, and 42.

Discussion

Stock Differentiation

Genetic results support the hypothesis that Foxe Basin and Baffin Bay walrus are different stocks. Within Foxe Basin walrus, our results do not support the hypothesis that Igloolik and Hall Beach walrus are genetically different. Similarly, within the Baffin Bay walrus, samples from Grise Fiord and Resolute Bay do not differ. There is a high degree of genetic overlap for microsatellite alleles between the two putative stocks (Figure 3) showing that stocks are characterized mainly by different proportions of alleles, not by different alleles. Haplotype diversity (48 haplotypes/139 individuals) may be so high that tests of genetic differentiation are not powerful. Nevertheless, Foxe Basin walrus are still differentiated from Baffin Bay walrus.

A non-nested AMOVA comparing 13 minor sample groups in Table 1 was also done, and in fact showed more significant differences at $p < 0.05$ among the minor sample populations than did the nested AMOVA. However, when table-wide comparison criteria were applied (Rice 1989), few differences were significant at the table-wide level. For haplotypes, the pattern of differences was scattered, and were for comparisons with Igloolik 1988 and Resolute Bay 1996, both small samples. These differences can be considered to be sampling artifacts. For microsatellites, 10/78 comparisons were significant at the table-wide level, and all 10 were Foxe Basin *versus* Baffin Bay comparisons. Thus in the nested analysis (Tables 2 and 3), Foxe Basin and Baffin Bay walrus differ.

Distances on the phylogenetic tree (Figure 1) are indicative of F_{st} values for the microsatellite AMOVA among the 13 sample populations. Baffin Bay and Foxe Basin samples are on separate branch clusters, and most distances within Baffin Bay samples and within Foxe Basin samples are closer to each other than to samples in the other putative stock. The longest inner branch separates Foxe Basin and Baffin Bay walrus. Igloolik 1988 is most likely on a more distant branch because there were only two individuals in the sample, thus this long branch may be due to chance.

A study by Andersen and Born (2000) demonstrated that walrus from northwestern Greenland, in the North Water polynia of northern Baffin Bay and Smith Sound, and walrus from west-central Greenland were genetically distinct subpopulations. Baffin Bay and northwestern Greenland samples are not compared in this manuscript, however, it may be possible since 8 of 10 microsatellite loci that Anderson and Born used are also used in this study.

Recent studies involving lead isotope signatures in walrus teeth revealed a difference between the walrus hunted from Igloolik and Hall Beach (Outridge and Stewart 1999, Outridge *et al.* this meeting). Although Igloolik and Hall Beach are not genetically distinct, this result shows that two groups separate to different feeding areas and breeding colonies in the summer. A slow rate of interbreeding may be the reasons for similar genetic characteristics.

In spite of overall heterozygote deficiency, the genetic diversity of walrus is high. Low genetic diversity is often associated with overharvesting, or with a population "bottleneck" in the past, that is, an event which reduced the population to very low numbers. It is probable that neither applies to walrus. Both walrus and bowhead whales have approximately 9 haplotypes/15

animals, while beluga and narwhal have between 3 and 7 haplotypes/15 animals (obtained by statistical subsampling, Maiers *et al.* 2001). Also, the microsatellite diversity may be slightly higher in walrus (0.65/15 animals) than in beluga (0.589 to 0.653/15 animals), narwhal (0.582 to 0.597/15 animals), and bowhead (0.501 to 0.524/15 animals) (Maiers *et al.* 2001).

Kin relationships

Most of the other genetic relationships described below are of interest because they describe social relationships already known to exist in walrus and because they allow us to evaluate the potential of genetic markers for future research. If kinship measurements are combined with field information, sex of individuals, and information about the social status of individuals, they can be informative. This was not done in this study because of limited knowledge of the samples collected and because sample sizes are small and not collected systematically.

The probability of identity is not only another statistic related to genetic diversity, but also tells us how useful the genetic markers might be in other scientific studies in which individuals are resample. With probabilities of identity in the 10^{-14} range for the 13 microsatellite loci, the existing suite of genetic loci would be quite sufficient for studies in which animals are resampled.

Parent-Offspring-Like Relationships (POLRs), accompanied by other information, can be used to identify true parent-offspring relationships. Whether observed POLR pairs are actually parent and offspring can be determined only by examining field information to see if the relationship makes sense and/or by taking population size and reproductive parameters into account. POLR probabilities were 0.0061 for Foxe Basin and 0.0141 for Baffin Bay. If the Foxe Basin stock consists of 4000 individuals, approximately 8,000,000 (4000*4000/2) pairwise comparisons of individuals can be made. Therefore 0.61% or 48,800 of these pairs will have a POLR. Of course, the effective number of pairs considered in any comparison is much smaller, particularly is if is assumed that females are highly philopatric (Andersen and Born 2000). If we were comparing only 100 mature female and 100 offspring in a population, the possible number of pairwise comparisons is reduced to 10,000. If haplotypes are used in the POLR testing, then 0.03115 % of these, or 3 pairs would be expected to have a POLR due to chance sampling. If a larger number is observed, we would most likely conclude parent-offspring pairs were sampled.

If more microsatellite loci were sampled, the probability of a chance POLR would be reduced, and the probability of identifying true parents and offspring would increase. On average, the probability of a POLR at a microsatellite locus is 0.7 ($= .01^{(1/13)}$).

It would be of interest to determine what types of mating could result in the pattern of distribution of homozygosities observed in Figure 3. The pattern of heterozygote deficiency could not be caused entirely by only a fraction of mature males reproducing, or by a large number of females half-sibs in harems, but must be caused by some degree of inbreeding. The relationship of heterozygosity to reproductive success is worth investigating. Foe example, Amos *et al.* (2001) suggest that, in grey seals, heterozygous males might be fitter and have more successful harems. Also they observed that half-siblings from the same mother tend to have different fathers, and suggest that weak immunotolerance of sperm from previous partners may exist. Such factors would maintain the overall genetic diversity of the population.

Patterns of relatedness among pairs of individuals (Figure 3) suggest that there may be groups of individuals within the larger Foxe Basin stock that are not strongly related to other groups. This was not caused by differences among the 9 minor sample populations within this stock (Table 2). We will not speculate about the causes of this distribution in view of the small sample size and biased sampling. However, such distributions of relatedness can be informative. By examining the distributions of relatedness of both males and females, and summer and winter walrus, Andersen and Born (2000) concluded that there was male-mediated gene flow between the two populations examined, whereas female-mediated gene flow was restricted. Closely related females were believed to be highly philopatric (Andersen and Born 2000). These conclusions are not inconsistent with our observations. It would be of interest to examine these processes in Canadian locations.

References

Allen, P.J., W. Amos, P.P. Pomeroy, and S.D. Twiss. 1996. Microsatellite variation in grey seals *(Halichoerus grypus)* shows evidence of genetic differentiation between two British breeding colonies. Mol. Ecol. 4: 653-662.

Amos, W., J. Worthington, and H. Kokko. 2001. Do female grey seals select genetically diverse mates? Anim. Behav. 62: 157-164.

Andersen, L.W. and E.W. Born. 2000. Indications of two genetically different subpopulations of Atlantic walruses (*Odobenus rosmarus rosmarus)* in west and northwest Greenland. Can. J. Zool. 78: 1999-2009.

Born, E.J., I. Gjertz and R.R. Reeves. 1995. Population assessment of Atlantic walrus *(Odobenus rosmarus rosmarus)*. Nor. Polarinst. Medd. 138:1-100.

Buchanan, F.C., L.D. Maiers, T.D. Thue, B.G.E. de March, and R.E.A. Stewart. 1998. Microsatellites from the Atlantic walrus *Odobenus rosmarus rosmarus*. Mol. Ecol. 7: 1083-1090.

Cavalli-Sforza, L. L. and A.W.F. Edwards. 1967. Phylogenetic analysis: Models and estimation procedures. Am. J. Human Genetics. 19: 233-257.

Cosens SE, R. Crawford, B.G.E. de March, and T.A. Shortt. 1993. Report of the Arctic Fisheries Scientific Advisory Committee for 1991/92 and 1992/93. Can. Man. Rep. Fish. Aquat. Sci. (2224: IV + 51 p.

Excoffier, L.; P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author, Department of Genetics, University of Washington, Seattle.

Guo, S. W. and E.A. Thompson. 1992. Performing the Exact Test of Hardy-Weinberg proportions for multiple alleles. Biometrics. 48: 361-372.

Haldane, J.B.S. 1954. An exact test for randomness of mating. J. Genet 53: 631-635

Hardy, G.H. 1908. Mendelian proportions in a mixed population. Science 28: 49-50.

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

Outridge, P.M. and R.E.A. Stewart. 1999. Stock discrimination of Atlantic walrus (*Odobenus rosmarus rosmarus)* in the eastern Canadian Arctic using lead isotope and element signature in teeth. Can. J. Fish. Aquat. Sci. 56: 105-112.

Paetkau D. and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology 3:489-95.

Raymond M. and F. Rousset. 1995. An exact test for population differentiation. Evolution 49: 1280-3.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 41: 223-225.

Robertson A, and W.G. Hill. 1984. Deviations from Hardy-Weinberg Proportions: Sampling Variances and use in Estimation of Inbreeding Coefficients. Genetics 107:703-18.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425.

Schneider, S.; J.M. Kueffer; D. Roessli, and L. Excoffier. 1997. Arlequin ver. 1.1: Asoftware for population genetic analysis. Geneva, Switzerland: Genetics and Biometry Laboratory, University of Geneva.

Weinberg, W. 1908. Über den Nachweis der Vererbung beim Menschen. Jahreshefte Verein. F. vaterl. Naturk. in Württenburg. 64: 368-382.

Weir, B.S., and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Weir, B. S. 1996. Genetic Data Analysis II. Sunderland, MA, USA: Sinauer Associates, Inc. 445 p.

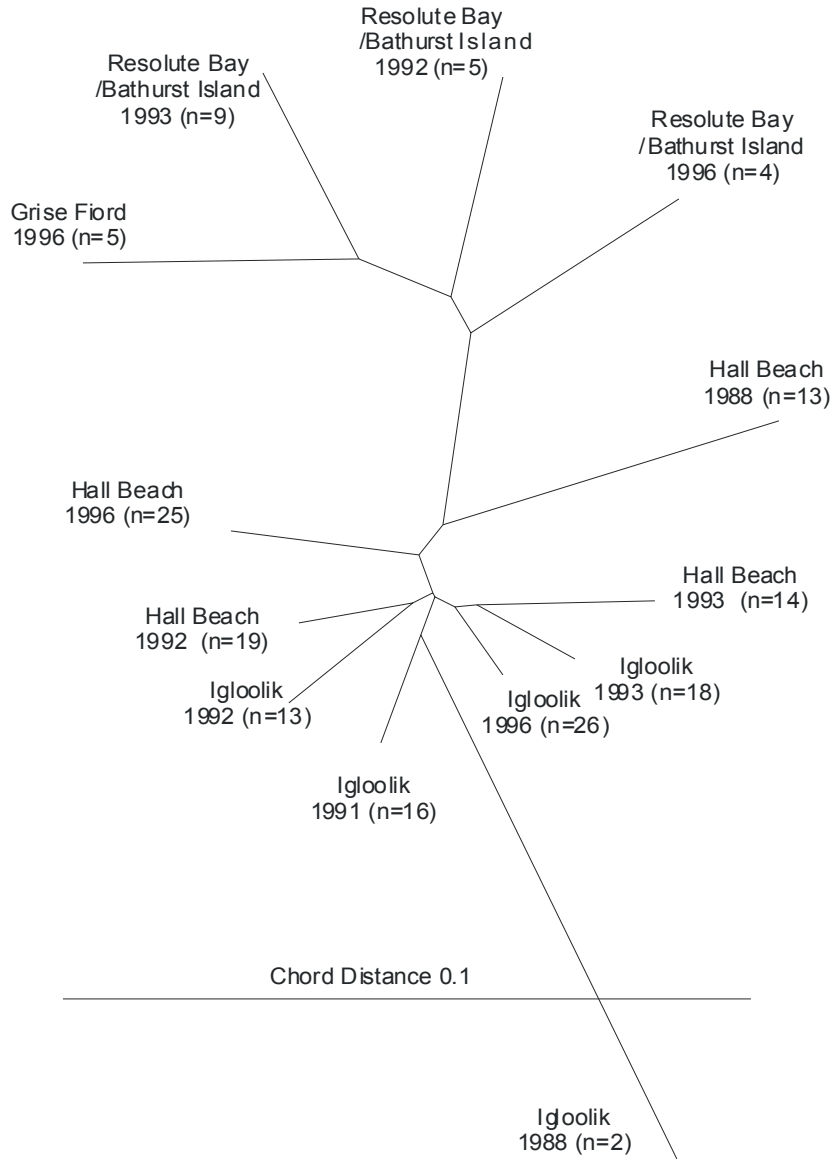

Figure 1. Unrooted tree diagram for 13 walrus sample populations in Table 1. The tree was constructed using Cavalli-Sforza's chord distance, calculated from population allele and haplotype frequencies, as a measure of distance between populations (Cavalli-Sforza and Edwards 1967). The neighbour-joining method of Saitou and Nei (1987) was used to construct the tree.

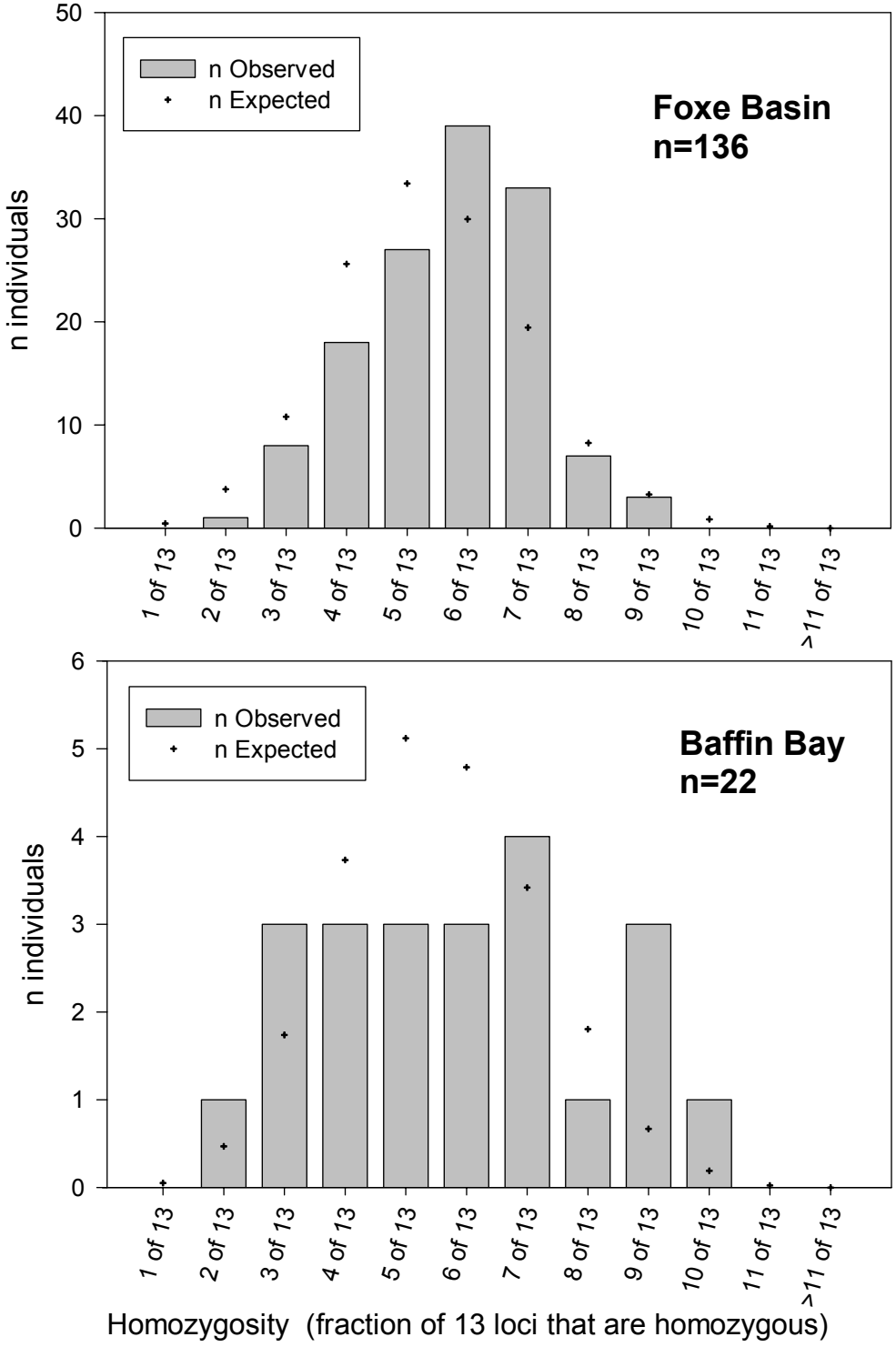

Figure 2. Observed and predicted frequencies of homozygous loci within individuals in two populations.

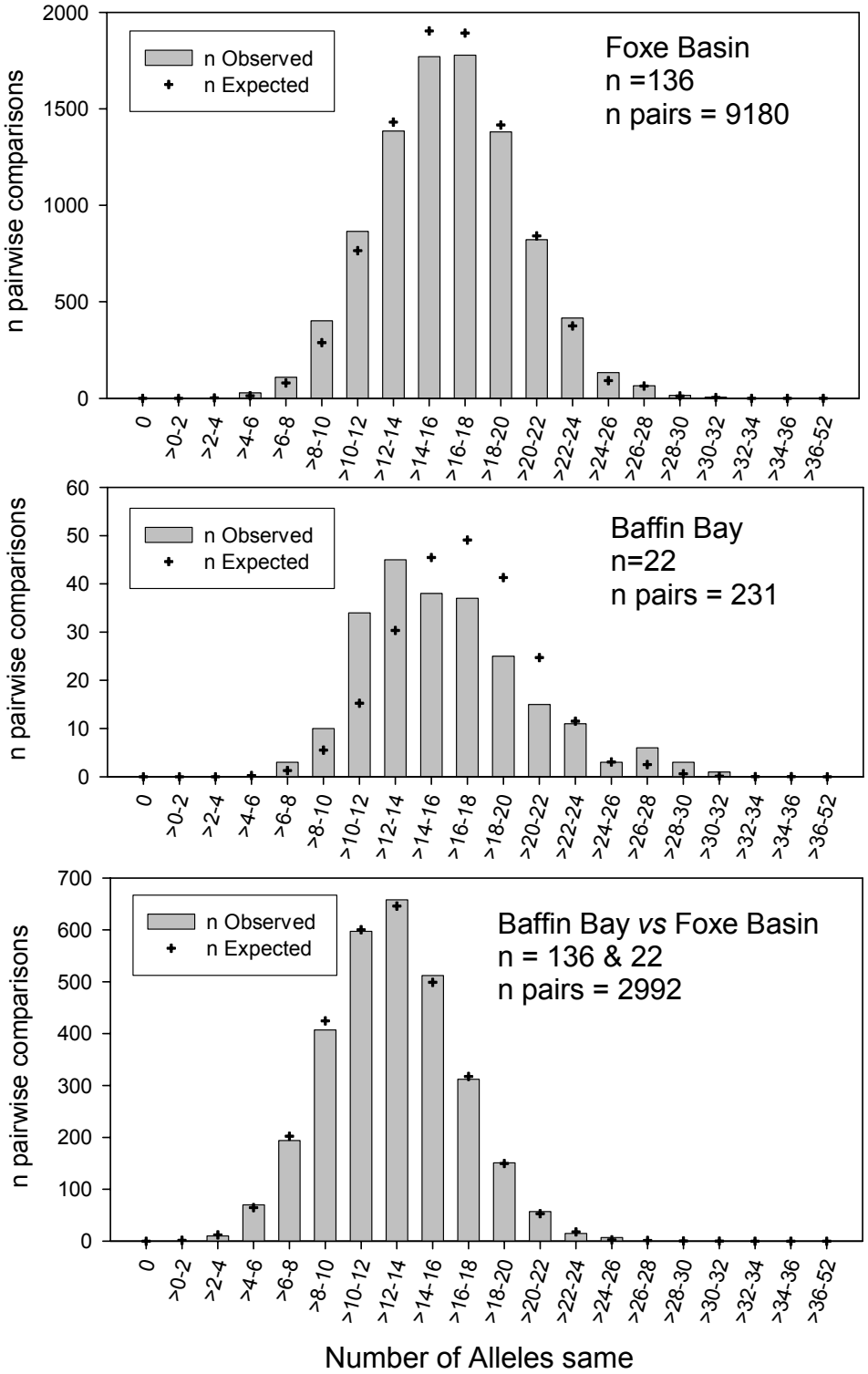

Figure 3. Observed and predicted number of alleles the same, of a possible 52 comparisons for each pair of individuals (13 loci x 4 comparisons/locus), in two populations.

Table 1. Walrus (4 major sample populations and 13 minor sample populations within these) used for genetic analyses.

Source	Year	n = number of walrus sampled	n samples with Haplotypes	n samples with Microsatellite data
Hall Beach	1988	13	12	3
	1992	19	16	19
	1993	14	13	14
	1996	25	22	25
	All Years	71	63	61
Igloolik	1988	2	1	2
	1991	16	15	16
	1992	13	11	13
	1993	18	16	18
	1996	26	21	26
	All Years	75	64	75
Grise Fiord	1996	5	4	4
Resolute/Bathurst	1992	5	4	5
	1993	9	3	9
	1996	4	1	4
	All Years	18	8	18
Total		169	139	158

Table 2. Nested AMOVA (Analysis of Molecular Variance) of microsatellite data.

Source	df	df breakdown	Sum of Squares (SS)	SS breakdown	Variance due to Source	Variance due to Source	% of total Variance	% of total Variance within Location	Probabilities of F - statistic	Interpretation
Among Groups	3		38.23		0.1415		3.40		0.0033	4 Locations differ
Hall Beach *vs* Igloolik		1		0.991		-0.1413		-0.35	0.8563	HB and IG do not differ
Grise Fiord *vs* Resolute/BI		1		5.008		0.0251		0.60	0.4985	GF and RB/BI do not differ
Foxe Basin *vs* Baffin Bay		1		32.229		0.3773		8.60	0.0010	Foxe Basin & Baffin Bay differ
Years within Groups	9		32.11		-0.0193		-0.46		0.9777	years within 4 locations do not differ
within Hall Beach		3		7.007		-0.5945		-1.52	1.0000	years within Hall Beach do not differ
within Igloolik		4		15.456		-0.0070		-0.17	0.7732	years within Igloolik do not differ
within Resolute Bay/Bathurst		2		9.946		0.0661		1.59	0.3568	years within RB/BI do not differ
Individuals in Populations	303		1222		4.0333		97.06		0.0000	There is variation within populations
within Hall Beach		118		469.54		3.9792		101.52		(trivial result)
within Igloolik		145		588.86		4.0611		100.17		
within Resolute Bay/Bathurst		33		134.69		4.0814		98.41		
within Grise Fiord		7		29		4.1429		100.00		
Total	315		1292		4.1555		100.00			

Table 3. Nested AMOVA (Analysis of Molecular Variance) of haplotype data.

Source	df	df breakdown	Sum of Squares (SS)	SS breakdown	Variance due to Source	Variance due to Source	% of total Variance	% of total Variance within Location	Probabilities of F - statistic	Interpretation
Among Groups	3		2.01		0.6703		1.44		0.0890	4 Locations differ
Hall Beach *vs* Igloolik		1		0.686		0.0028		0.59	0.1202	HB and IG do not differ
Grise Fiord *vs* Resolute/BI		1		0.292		-0.0408		-10.00	0.7459	GF and RB/BI do not differ
Foxe Basin *vs* Baffin Bay		1		1.033		0.0252		5.06	0.0088	Foxe Basin & Baffin Bay differ
Years within Groups	9		4.51		0.0032		0.66		0.1996	years within 4 locations differ slightly
within Hall Beach		3		1.941		0.0155		3.25	0.0323	years within Hall Beach differ
within Igloolik		4		1.615		-0.0067		-1.40	0.9198	years within Igloolik do not differ
within Resolute Bay/Bathurst		2		0.958		0.0404		9.52	0.3290	years within RB/BI do not differ
Individuals in Populations	116		54.6		0.4704		97.89		0.0372	There is variation within populations (trivial result)
Total	128		61.1		0.4805		100.00			

Table 4. Patterns of microsatellite and haplotype differentiation among samples from 4 major locations. F_{st} values for haplotype comparisons are above the diagonal, F_{st} values for microsatellelite comparisons are below the diagonal. Bolded microsatellite comparisons are significant at $p < 0.0000$, bolded haplotype comparisons are significant at $p < 0.05$. For all other comparisons, $p > 0.05$.

Locations	Hall Beach	Igloolik	Grise Fiord	Resolute Bay / Bathurst Island
n with haplotypes	63	64	4	8
n with microsatellite loci	61	75	4	18
			Haplotypes	
Hall Beach		0.0086	0.00094	**0.06321**
Igloolik	-0.00562		-0.00476	**0.06199**
Grise Fiord	**0.10152**	**0.11668**		-0.06667
Resolute Bay/Bathurst Island	**0.0723**	**0.08661**	0.01604	
		Microsatellites		

Table 5. Characteristics of microsatellite loci and haplotypes in 4 sample populations. H_o= observed heterozygosity, H_e= expected heterozygosity (or 1 - genetic diversity) based on sample population allele frequencies, F_{is} = inbreeding coefficient, ID_e = the probability of sampling two identical alleles. Loci with F_{is} values marked with an asterisk (*) are at Hardy-Weinberg equilibrium.

	All Data		Hall Beach				Igloolik				Grise Fiord				Resolute/Bathhurst			
Microsatellite Locus	n alleles	ID_e	n alleles	H_e / H_o	F_{is}	ID_e	n alleles	H_e / H_o	F_{is}	ID_e	n alleles	H_e / H_o	F_{is}	ID_e	n alleles	H_e / H_o	F_{is}	ID_e
Orrfcb2	4	0.135	4	0.69 / 0.61	0.310*	0.155	4	0.79 / 0.62	0.563*	0.142	2	1.00 / 0.38	1.000*	0.391	3	0.72 / 0.64	0.587*	0.128
Orrfcb3	8	0.073	7	0.28 / 0.72	-0.009	0.081	6	0.32 / 0.73	0.040	0.070	3	0.25 / 0.66	-0.028	0.118	5	0.22 / 0.72	-0.004	0.798
Orrfcb7	13	0.018	12	0.23 / 0.85	0.036	0.212	13	0.15 / 0.87	0.005	0.017	5	0.00 / 0.78	-0.125	0.048	7	0.22 / 0.79	0.029	0.043
Orrfcb9	6	0.139	5	0.42 / 0.61	0.052	0.154	5	0.45 / 0.61	0.296	0.153	3	0.50 / 0.53	0.050	0.220	4	0.19 / 0.65	-0.121	0.121
Orrfcb11	7	0.168	6	0.39 / 0.62	0.096*	0.148	6	0.38 / 0.62	-0.015	0.146	2	0.75 / 0.22	-	0.610	4	0.61 / 0.38	-0.044	0.391
Orrfcb16	11	0.032	9	0.18 / 0.82	0.003	0.034	9	0.21 / 0.79	0.026	0.043	3	0.25 / 0.59	-0.125	0.165	7	0.35 / 0.80	0.129	0.041
Orrfcb21	10	0.048	9	0.27 / 0.76	0.160	0.059	9	0.28 / 0.76	0.081	0.571	4	0.25 / 0.66	-0.028	0.118	7	0.06 / 0.81	0.033*	0.037
Orrfcb23	19	0.011	16	0.15 / 0.89	0.059	0.013	19	0.21 / 0.90	0.013	0.010	4	0.25 / 0.66	-0.028	0.118	7	0.44 / 0.81	0.280*	0.034
Orrfcb24	8	0.042	8	0.24 / 0.81	0.026	0.038	7	0.26 / 0.77	-0.020	0.053	2	0.50 / 0.50	0.167	0.250	5	0.50 / 0.65	0.189*	0.120
hg4.2	9	0.229	7	0.54 / 0.47	-0.002	0.228	8	0.49 / 0.47	0.087	0.281	3	0.00 / 0.62	-0.417	0.144	7	0.39 / 0.68	0.101	0.100
hq8.1	6	0.244	4	0.55 / 0.41	-0.052	0.345	5	0.55 / 0.47	-0.007	0.282	4	0.40 / 0.58	-0.028	0.176	3	0.59 / 0.53	0.134	0.225
hq6.1	4	0.325	4	0.67 / 0.41	0.195	0.350	4	0.57 / 0.43	-0.096	0.319	2	0.40 / 0.42	-0.533	0.336	4	0.61 / 0.41	0.055	0.353
pv9	4	0.153	4	0.28 / 0.62	-0.058	0.141	4	0.41 / 0.51	-0.169	0.244	3	0.20 / 0.54	-0.183	0.212	4	0.39 / 0.60	-0.067	0.160
Total	109		95				99				40				66			
Mean All Loci	8.385	0.124		0.377 / 0.66	0.037	0.151		0.388 / 0.658	0.020	0.179		0.365 / 0.548	-0.116	0.224		0.408 / 0.651	0.024	0.196
Joint Probabilities		8.0E-15				2.8E-14				3.0E-14				3.1E-10				1.7E-13
Haplotypes	48	0.016 $=ID_{eh}$	25			0.068 $=ID_{eh}$	28			0.056 $=ID_{eh}$	4			0.25 $=ID_{eh}$	4			0.281

Table 6. Descriptive Statistics for Foxe Basin and Baffin Bay walrus samples.

	Foxe Basin	Baffin Bay
n walrus	146	23
n haplotyes	44	6
ID_{eh} = haplotype probability of identity = haplotype POLR	0.0513	0.2222
n microsatellite alleles	106	69
H_e = microsatellite genetic diversity	0.6615	0.6494
ID_e = microsatellite probability of identity	2.40E-14	1.91E-10
microsatellite probability POLR	0.006072	0.014115
Haplotype and Microsatellite data combined		
ID_e = probability of identity	1.23E-15	4.24E-11
probability POLR	0.0003115	0.003358