

The combined use of organochlorine contaminant profiles and molecular genetics for stock discrimination of white whales (*Delphinapterus leucas*) hunted in three communities on southeast Baffin Island

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ABSTRACT

Putative stock differences in white whales (*Delphinapterus leucas*) landed by hunters between 1992 and 1996 from the southeast Baffin Island communities of Kimmirut (KI), Iqaluit (IQ) and Pangnirtung (PA) were examined using organochlorine contaminant (OC) profiles of 124 whales, the molecular genetics of 270 whales and both types of data from 97 whales. OC concentrations were generally lower in whales hunted in PA than those hunted in KI and IQ, and many OCs were lower in KI than IQ. In canonical discriminant function (CDA) using 13 OC predictor variables (10 OC groups, mirex, octachlorostyrene and endosulfan), the first canonical function accounted for 77% of the variance and separated whales from PA with those from IQ and KI; the second canonical function separated whales from KI with those from IQ. A previous study of the molecular genetics of white whales showed that whales hunted in the three communities were significantly differentiated on the basis of haplotype and/or microsatellite allele frequencies (de March *et al.*, 2002).

When the results of two studies were combined, many whales were slightly more strongly associated with a particular source hunting community than they were in the component studies. Using *a posteriori* crossvalidation probabilities in an analysis with variables from both studies, 72% of white whales were correctly crossvalidated to their source hunting community; 82.5% from PA; 56.5% from IQ; and 58.8% from KI. The highest misclassification rates were KI to IQ (23.5%), IQ to KI and IQ to PA (21.7% in both cases) and the lowest rates were PA to KI (3.5%), PA to IQ (14.0%) and KI to PA (17.6%). This pattern of assignments was not significantly different from those in the genetics or contaminants studies alone. However, the crossvalidation probabilities to the most likely source communities were approximately 20% larger in the combined analysis than in the component studies. Canonical scores in the combined analysis were more strongly correlated with variables from the OC Study than with variables from the genetics study. Whales placed to PA and IQ could be identified primarily by their OC signatures, however many whales from PA also had a strong PA genetics signature. Whales from IQ were identifiable only by their OC signatures. Both a strong KI genetics and OC signature described approximately half of whales from KI. We believe that at least three stocks were sampled from the three communities.

Some whales in PA were very distinct, confirming previous beliefs that a separate stock occurs in Cumberland Sound. Whales hunted in IQ and KI differed to a lesser degree, and may be from stocks subject to a gradient or from a mixture of stocks. Some whales from PA are more likely to have genotypes and OC signatures that are also found in IQ and KI than the reverse. It is possible that summering areas of the stocks that were identified in KI and IQ are not consistent from year to year or across generations.

The main problems in combining results for individuals used in several studies, particularly when there are many measurements for relatively few individuals, is to find a limited number of relevant predictor variables that can be used in the combined analysis, while avoiding both overparameterisation and results blurred by meaningless variables.

KEYWORDS: GENETICS; ORGANOCHLORINES; DISTRIBUTION; MIGRATION; ARCTIC; NORTH AMERICA

INTRODUCTION

The southeast Baffin white whale (*Delphinapterus leucas*) stock was once defined as the whales summering near southeast Baffin Island and caught by hunters from Pangnirtung (hunted in Cumberland Sound), Iqaluit (formerly Frobisher Bay, hunted in Frobisher Bay) and Kimmirut (formerly Lake Harbour, hunted on the southeast coast of Baffin Island) (Sergeant and Brodie, 1975; Richard and Orr, 1986; Richard, 1991; Bodaly *et al.*, 1992) (Fig. 1). It is now believed that several stocks are hunted in this area although the stock boundaries are not clear. It is important to understand whether or not southeast Baffin (SEB) communities hunt the same stocks of white whales so that appropriate management decisions can be made.

In Kimmirut (KI), white whales are hunted mainly during the spring and autumn migrations. It is hypothesised that they are whales originating from Hudson Bay summer aggregations, and that they overwinter in the Hudson Strait and its open waters. Hunters from KI believe they hunt a 'local' stock in spring and summer, and migrating animals in the autumn, the same animals that migrated past Coral Harbour one week earlier (P. Richard, pers. comm.). Thus

some white whales that are hunted from KI may also be hunted in summering areas in Hudson Bay and northern Québec (Reeves and Mitchell, 1989; Richard *et al.*, 1990).

The stock identity of white whales from Iqaluit (IQ) is unclear. Unlike in KI, whales are hunted in Frobisher Bay all summer, whenever they become available. It is possible that these whales are the remnants of a reduced summering stock (G. Williams, pers. comm.) or they may be summer wanderers from northern Québec or from offshore areas.

There is considerable evidence supporting the hypothesis that at least some white whales hunted in Cumberland Sound are a separate stock. Hunters from IQ and KI have always believed that they do not hunt the same white whales as Pangnirtung hunters because of differences in migration times and adult sizes (Southeast Baffin Beluga Review Committee (SEBBRC), 1991; Planning Committee for the Co-management of Southeast Baffin Beluga, 1994; Department of Fisheries and Oceans (DFO), 2002). In fact, hunters from PA report that there are three different white whale groups that come into Cumberland Sound that can be distinguished by their appearance, size, health, taste, texture of maktaq and behaviour (Kilabuk, 1998). White whales hunted from PA and IQ are on average larger at a given age

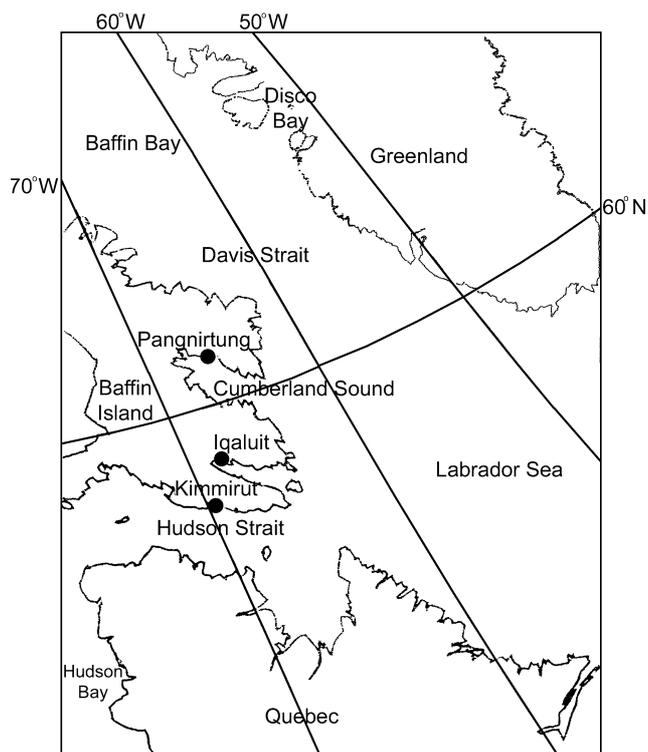


Fig. 1. Study area.

than those from KI (de March, unpublished data). In addition, recent radio-tagging work by Richard (pers. comm.; Department of Fisheries and Oceans (DFO), 2002) showed that Cumberland Sound white whales stay in the northeast sector of Cumberland Sound in the winter. The white whales that congregate near the Ranger River at Clearwater Fiord on Cumberland Sound were listed 'endangered' by COSEWIC in 1989 (Campbell, 1989) after considerable declines in this area. With this and genetics evidence (see below), quotas were lifted for IQ and KI in 1999, whilst the quota was maintained for PA (Richard, 1991). Since that time, attempts have been made to obtain more information on all SEB stocks.

Genetic results support ecological knowledge about whales hunted in SEB communities. De March *et al.* (2002) examined a maternally-inherited d-loop mitochondrial DNA (mtDNA) sequence of 324 nucleotides with 22 variable positions and 15 nuclear microsatellite loci in white whales from the three SEB communities and from several high Arctic locations. Twenty of 55 haplotypes found in North America were found in SEB. On the basis of mtDNA haplotypes, white whales hunted in KI were not significantly differentiated from those hunted in IQ (lesser F_{st} , $p=0.44$), but whales from both IQ and KI were significantly differentiated from those hunted in PA (lesser F_{st} , both $p<0.00$) (de March *et al.*, 2002). Whales from PA had more uncommon and unique haplotypes than those from IQ and KI. Several haplotypes from PA whales occurred only in the North American western Arctic, while those from KI and IQ mostly resembled those from western and northern Hudson Bay (de March and Postma, 2003). In addition, whales hunted in the three SEB communities were significantly differentiated from each other on the basis of allele frequencies at 15 microsatellite loci. Whales from PA differed strongly from those from KI ($p<0.00$), and whales from IQ did not differ from those from KI or PA (both $p=0.01$, not significant at a table-wide level) (de March *et*

al., 2002). In a dendrogram using microsatellite loci, whales from the three communities clustered on one branch that was distant from other high Arctic locations (de March *et al.*, 2002). In another comparison involving samples from KI, northern Québec and Hudson Bay locations, KI samples most resembled those from northern Québec (de March and Postma, 2003). There were also temporal differences among years within communities for both types of loci, though these were smaller than differences among the three locations (de March *et al.*, 2002). This observation also supports the hypothesis that several stocks exist, even if not strongly delineated.

Organochlorine contaminants have been used to determine stock affiliations of whales in univariate and multivariate analyses (Aguilar, 1987; Aguilar *et al.*, 1993; Stern *et al.*, 1994; Krahn *et al.*, 1999; Innes *et al.*, 2002). Different patterns of organochlorine contaminant (OC) concentrations in marine mammals are caused by differences in feeding. They may thus reflect a number of factors including: different prey species or proportions of these in the diet; the trophic status of prey species; feeding patterns in summering and wintering areas and/or on migration routes; differential feeding behaviour of different social groups etc. An analysis of eastern North American white whales using OC data showed that there were strong differences in OC concentrations among samples from Greenland, Grise Fiord, PA, KI and several Hudson Bay locations (Innes *et al.*, 2002). This study showed significant differences in OC concentrations between whales hunted in the SEB communities of PA and KI ($n=7$ whales from PA, $n=15$ from KI). Of 64 OCs used in the study, 33 were significantly different ($p<0.05$) between PA and KI samples, and concentrations were higher in KI than in PA samples for 24/33 OCs. Differences were not as large as differences across larger geographic distances. Ten of 15 white whales from KI and 5 of 7 from PA were crossvalidated to their source hunting community in canonical discriminant function analysis (CDA). The remaining 7/22 from PA and KI were crossvalidated to the Belcher Islands (southern Hudson Bay). None were misclassified to hunting locations in Greenland or Grise Fiord in the Canadian High Arctic (Innes *et al.*, 2002).

It is generally assumed that the results of more than one type of study using the same subjects will yield more information about stock differences than one study alone (Donovan, 1991; IWC, 2002). For this study, both genetics information and OC data were available from many white whales, thus providing the opportunity to investigate the SEB stock question further.

METHODS

Samples

Lower jaws, blubber and skin samples were obtained from white whales caught by hunters from Kimmirut (KI), Iqaluit (IQ) and Pangnirtung (PA) between 1982 and 1996 (Table 1). Blubber samples were frozen and stored at -20°C until organochlorine contaminant (OC) analyses were undertaken in 1995. The ages of the whales sampled were determined by counting annuli on sagittal thin sections of the second or fifth tooth, whichever had the least wear (Perrin and Myrick, 1980; Goren *et al.*, 1987; Wainwright and Walker, 1988; Brodie *et al.*, 1990). White whales ≤ 2 years of age were not used in the OC analyses. Laboratory sex determinations were conducted using the methods described in Bérubé and Palsbøll (1996).

Table 1
Locations, years and numbers of samples. A total of 297 white whales (270 from the genetics study and 124 from the OC study with 97 overlapping) were sampled.

Location and year	<i>n</i> genetics study	Sex ratio F:M	<i>n</i> OC study	Sex ratio F:M	Mean age F	Mean age M	<i>n</i> joint study
Pangnirtung (PA)							
1982	10	1:9	6	0:6		8.3	6
1985	2	0:2					
1986	20	5:18	20	4:16	3.5	5.8	20
1991	10	4:06					
1992	20	7:14	20	7:13	14.8	12.3	17
1993, 1994	26	13:19					
1995	17	9:8	17	9:8	6.6	13.7	14
1996	17	10:11					
Location total	122		63				57
Iqaluit (IQ)							
1984, 1989	21	7:15					
1992	18	9:9	12	6:6	6.7	4.8	12
1993, 1994	31	3:21					
1996	13	4:7	12	2:10	5.5	6.3	11
Location total	83		24				23
Kimmirut (KI)							
1992	22	11:11	28	13:15	10.7	9.3	17
1993, 1994	31	19:14					
1995	8	3:6	6	2:4	4.5	17.0	0
1996	4	1:3	3	1:2	7.5	5.8	0
Location total	65		37				17
	270		124				97

Genetic analyses

The whales used in the genetic analyses (Table 1) were the same 270 whales described in de March *et al.* (2002) that had both haplotype and microsatellite data. Of these, 97 whales were also used in the OC study. An mtDNA sequence of 324 nucleotides found near the beginning of the d-loop and 15 microsatellite loci were examined (de March *et al.*, 2002).

For the study presented here, probabilities were calculated of every individual's genotype, treated as an unknown, occurring in different sample populations. This was done to produce summary descriptions for all individuals that could be used in the analysis of both studies combined. For this calculation, population allele frequencies of '0' were reset to 0.5, as Waser and Strobeck (1998) suggest, to ensure that individuals with unique alleles or haplotypes would have positive probabilities of occurring in all populations. Individual whales were then 'assigned' to a most likely sample population of origin on the basis of this probability (Waser and Strobeck, 1998; Paetkau *et al.*, 1995). The probabilities of an individual's genotype occurring in different populations were standardised to add up to 1 by applying Baye's formula, and these assignment probabilities were used in the CDA analysis combining the two studies. Assignment calculations were done using in-house software using Visual Basic.

Genetic diversities in whales hunted in the three communities, not previously described, were calculated as 'rarefied' values of $D_i = 1 - \sum_u (p_{iu})^2$ for haplotypes and as $D = 1 - \sum_l \sum_u (p_{lu})^2/m$ for microsatellites, where p_{iu} is the frequency of the u th allele at the l -th locus, and m is the number of loci (Weir, 1996). The 'rarefied' values, which were calculated with 1,000 sub-samples without replacement of 15 white whales (Hurlbert, 1971), are not expected to be correlated with sample size.

Chemical analysis

A total of 124 samples from the three communities were extracted and analysed in random batches so that observed differences between sampling sites could not be attributed to

any systematic analytical variation. All laboratory analyses were performed using the same methodology, instrumentation and analyst over a period of two years. Other quality assurance measures included the analysis of standard reference materials (NIST cod liver oil 1588) and duplicated analysis of every 12th sample. The duplicate results were satisfactory, and results were averaged for the duplicated analyses.

Determinations of OCs in white whale blubber tissues followed the procedures described by Stern *et al.* (1994). Blubber samples were partially thawed and 2g was combined with anhydrous Na₂SO₄ (heated at 600°C for 16 hours prior to use). The mixture was then extracted twice with hexane in a small (50ml) ball mill, with the hexane decanted between extractions. Surrogate recovery standards of PCB30 and octachloronaphthalene (OCN) were added prior to extraction. Extractable lipids were determined gravimetrically on a fraction (1/10) of the extract. A portion of the extract equivalent to approximately 100mg lipid was separated into three fractions of increasing polarity on Florisil (8g; 1.2 % v/w water deactivated). The first fraction was eluted with hexane and contained PCBs, DDE, *trans*-nonachlor and mirex; the second fraction was eluted with hexane:DCM (85:15) and contained HCHs, most chlorinated bornanes, chlordanes and most DDTs. Some chlorobornanes, most notably T2 (Parlar no. 26), were partially eluted with hexane. The third fraction, containing dieldrin and heptachlor epoxide, was eluted with a 1:1 mixture of hexane:DCM. After addition of aldrin as a volume corrector, each fraction was analysed for OCs by capillary gas chromatography (GC) with ⁶³Ni electron capture detection (ECD) by means of an automated Varian 3400 GC (Varian Instruments, Palo Alto, CA). Samples were injected on a 60mm × 0.25mm i.d. DB-5 column (film thickness=0.25µm). H₂ was used as the carrier gas (2mL/min) and N₂ as the make-up gas (40mL/min). A total of 103 PCB congeners (including co-eluting congeners) and 40 OC pesticides were quantified by using external standard mixtures (Ultra Scientific, North Kingstown, RI).

Recoveries of the surrogates, PCB30 and OCN were uniformly greater than 90% and no corrections were made for recoveries. One hundred and thirty-three (OC) compounds, some co-eluded, were quantified. Of these, 88 had consistent non-zero values and were kept for statistical analyses. All data are in ug/g or ppb in wet blubber weight (approximately 80% lipid).

OC data were statistically corrected for covariates before they were used in multivariate analyses, as suggested by Tabachnick and Fidell (2001). Specifically, the model:

$$\log(\text{concentration}) = a_s \times \text{sex} + b \times \text{age} + c_s \times \text{sex} \times \text{age} + d \times \text{location} \quad (1)$$

in which *sex* (= M or F) and *location* (= IQ, KI or PA) are class variables; *age*, a continuous variable describing age in years; a_s , the sex effect; d_l the location effect; b the coefficient describing the effect of *age*; and c_s , coefficient of the *sex* × *age* effect for each sex, was first used to describe every OC and OC group using the general linear models procedure (PROC GLM) in SAS Inst. Inc. (1989). When OC groups such as ΣDDT or Σ7-CB were used, component concentrations were summed before using the logarithmic transformation. Raw logged values for each OC were then adjusted for covariates with coefficients from Equation (1) as follows assuming an age of 10 years and male sex:

$$\log(\text{concentration})_{\text{adjusted}} = \log(\text{concentration})_{\text{observed}} - a_s \times \text{sex} - b \times \text{age} - c_s \times \text{sex} \times \text{age} \quad (2)$$

Because of the covariate correction, results for CDA are not expected to correlate with age or sex. The values and partial probabilities (Type III error) of the four effects above and of the three contrasts comparing locations pairwise (Iqaluit versus Pangnirung = IQ – PA), (KI – PA) and (IQ – KI), were also calculated.

All statistical analyses of OC data, for 124 individuals in the OC Study and for the 97 used in both studies, were undertaken using various linear models programs available in SAS (Statistical Analysis System, SAS Inst. Inc., 1989). OC concentration patterns among sampling locations were described using Canonical Discriminant Analysis (CDA) with the PROC DISCRIM and PROC STEPDISC procedures (SAS Inst. Inc., 1989). The probabilities of population memberships were obtained by ‘crossvalidating’ all individuals (Option CROSSLIST in PROC DISCRIM in SAS Inst. Inc., 1989). In crossvalidation, the individual to be tested is removed from the data, the canonical functions are calculated without this individual, and then the individual is placed with the functions from the reduced dataset (Lachenbruch and Mickey, 1968).

In view of concerns about overparameterisation and lack of power (Tabachnick and Fidell, 2001; also see Discussion), we performed the presented CDA with a limited number of predictor variables. The 13 predictor variables were: ΣDDT (o,p’- and p,p’-DDT); ΣDDE; ΣDDD; ΣHCH; ΣCHL; Σ4-CB (tetrachlorobiphenyls); Σ5-CB; Σ6-CB; Σ7-CB; Σ8-CB; mirex; endosulfan; and octachlorostyrene (Table 2). This number was considered to be few enough to avoid overparameterisation with the smallest sample size of 17 for KI. The results of stepwise CDA with these 13 predictor variables and stepwise CDA using all 88 OCs were also examined.

Statistical methods for combining results of two studies

Ninety-seven individual whales’ probabilities of being identified as originating from KI, IQ or PA, in both the Genetics and the OC Study were used as predictor variables in the CDA of results from both studies (the Joint Study).

The term ‘Genetics Placement Probability’ (GenPP) will be used to describe the probabilities of assignment to each of three source communities in the Genetics Study and ‘Organochlorine Contaminants Placement Probability’ (OCPP), to describe the crossvalidation probabilities to communities in the OC Study. The term ‘Joint Placement Probability’ (JointPP) will be used to describe the three probabilities of assignment derived from CDA in the Joint Study.

All assignment probabilities (GenPPs and OCPPs), and crossproducts of these probabilities for 97 whales were examined using CDA (PROC DISCRIM, SAS, Statistical Analysis System, SAS Inst. Inc., 1989). Although this produces 21 predictor variables, only 10 are linearly independent. The CDA produced three JointPPs for each of 97 individuals, one to each source community. Individuals were then crossvalidated to a community on the basis of this probability (Option CROSSLIST in PROC DISCRIM in SAS Inst. Inc., 1989).

RESULTS

Molecular genetics

Actual probabilities of assignments to the source hunting communities in the genetics study can be identified in Fig. 2. Of 270 white whales in the genetics study, 74 of 122 (61%) from PA, 42 of 83 (51%) from IQ and 31 of 65 (48%) from KI were assigned to their source hunting community (Table 3). Misassignments were primarily between KI and IQ (31% both ways). Misassignments between PA and either IQ or KI were lower, ranging from 18–22%. Patterns of assignments and misassignments were not significantly different between these 270 whales and the subset of 97 used in both studies (comparison of columns 1 and 3 in Table 3, Chi Squared=10.22, $p=0.2498$, 8 df). In the study with 97 whales, individuals hunted in PA had a higher probability of being assigned to their source community than individuals from KI or IQ (column 3b, Table 3).

In whales from PA, 6.3 ± 1.2 (mean \pm SD of rarefied values) haplotypes/15 whales was observed, while 5.0 ± 1.1 IQ and 4.1 ± 0.9 in KI. Actual numbers of haplotypes observed were 13, 9 and 7. Rarefied haplotype diversities for 15 individuals were 0.713 ± 0.09 for PA, 0.594 ± 0.11 for IQ and 0.527 ± 0.11 for KI. The number of microsatellite loci and microsatellite diversity did not differ notably in the three communities. The rarefied numbers of alleles for PA, IQ and KI were 80.0 ± 3.4 , 82.2 ± 5.0 and 80.4 ± 3.4 respectively, while diversities were 0.645 ± 0.01 , 0.648 ± 0.02 and 0.648 ± 0.02 .

OC Contaminants

The R^2 values for univariate OC analyses of covariance (Equation 1) were consistently higher with log-transformed than with untransformed data (Mean $r^2=0.336$ versus mean $r^2=0.303$). The *sex* × *age* interaction was significant at $p \leq 0.05$ for 67/88 OCs, while age was in 20/88 and sex in 0/88. The coefficient of the *sex* × *age* interaction was positive for all OCs, and the age coefficient was always smaller in magnitude and usually negative.

Among 88 OCs examined, 72 had significant location effects at $p \leq 0.05$. Significant probabilities ranged from 4.73×10^{-2} for PCB28 to 3.48×10^{-12} for PCB187 (Table 2). When table-wide statistical criteria were applied as a sequential von Bonferroni test (Rice, 1989), yielding a minimum significance level of $p=0.0004$ for a table-wide $\alpha=0.05$, 52% (46/88) of OCs still had significant location

Table 2

Statistics for selected organochlorine contaminants (OC) for white whales hunted in three communities on southeast Baffin Island. Mean concentrations and CVs are from corrected values from Equation (2). Other descriptions are from Equation (1), and include R² values, significance of covariates at $p \leq 0.05$ indicated as a 'y', significance of overall location effects and of contrasts comparing OC concentrations from the three communities pairwise. Sign of contrasts are designated by + or -, and contrasts not significant at $p \leq 0.05$ are bracketed. 'Trend' represents order of mean concentrations in white whales from the three communities.

Contaminant or contaminant group	Mean ng/g Kimmirut	CV (%)	Mean ng/g Iqaluit	CV (%)	Mean ng/g Pangnirtung	CVEquation (%)	Covariates [1] R ²	Covariates significant?	Probability of stock effects	Contrast			Trend
										IQ-KI	KI-PA	IQ-PA	
Congener and isomer groups													
∑3-CB	100.68	35	91.36	27	84.91	34	0.368	y	3.56E-02	(-)	+	(+)	KI>IQ>PA
∑4-CB	611.51	35	701.63	40	506.65	37	0.388	y	1.99E-03	(+)	+	+	IQ>KI>PA
∑5-CB	1510.76	39	1639.22	30	1088.58	35	0.479	y	8.44E-07	(+)	+	+	IQ>KI>PA
∑6-CB	1905.37	41	2408.73	40	1403.26	36	0.475	y	9.85E-08	+	+	+	IQ>KI>PA
∑7-CB	681.04	38	839.61	37	460.74	36	0.506	y	1.77E-10	+	+	+	IQ>KI>PA
∑8-CB	54.15	37	75.11	41	48.57	39	0.331	y	1.72E-04	+	(+)	+	IQ>KI>PA
PCB05 (2-CB)	209.37	63	136.33	43	142.12	60	0.179		3.77E-03	-	+	(+)	KI>IQ>PA
PCB207 (9-CB)	4.67	55	5.88	50	4.00	79	0.210		4.13E-03	(+)	+	+	IQ>KI>PA
∑PCB (all above)	5074.76	36	5882.73	35	3738.87	33	0.481	y	2.67E-07	(+)	+	+	IQ>KI>PA
∑DDT	2724.42	37	3051.93	42	1913.31	41	0.423	y	3.15E-05	(+)	+	+	IQ>KI>PA
∑DDE	826.87	35	868.38	34	655.75	38	0.417	y	5.39E-04	(+)	+	+	IQ>KI>PA
∑DDE	2449.99	44	2735.58	59	1847.96	39	0.360	y	4.08E-03	(+)	+	+	IQ>KI>PA
∑DDs (all above)	6018.89	35	6662.16	43	4414.75	36	0.432	y	2.36E-06	(+)	+	+	IQ>KI>PA
∑CHL	2322.73	33	2195.95	33	1739.30	34	0.407	y	2.27E-04	(-)	+	+	KI>IQ>PA
∑CBz	532.62	45	570.52	45	424.47	41	0.364	y	1.30E-02	(+)	+	+	IQ>KI>PA
∑HCH	281.58	31	243.40	32	229.83	36	0.281	y	1.37E-02	(-)	+	(+)	KI>IQ>PA
∑CHB	10,472.81	31	10,479.83	34	9119.43	31	0.390	y	7.62E-02	(-)	+	(+)	KI>IQ>PA
Endosulfan	14.06	95	17.18	56	8.78	74	0.255		1.81E-06	(+)	(-)	+	IQ>KI>PA
Octachlorostyrene	3.63	68	6.31	57	4.04	53	0.285		1.67E-03	+	(-)	+	IQ>KI>PA
Mirex	14.23	39	15.82	49	8.81	59	0.367		1.23E-08	(+)	+	+	IQ>KI>PA
Dieldrin	573.73	33	646.21	48	528.10	38	0.308	y	2.64E-01	(+)	(+)	(+)	IQ>KI>PA
Individual OCs of interest													
PCB187	195.84	45	267.37	37	119.18	54	0.498	y	3.48E-12	+	+	+	IQ>KI>PA
PCB193	25.67	51	22.59	30	13.40	57	0.435	y	1.19E-10	(-)	+	+	KI>IQ>PA
PCB132	81.50	47	120.71	48	57.39	45	0.460	y	1.32E-09	+	+	+	IQ>KI>PA
PCB185	10.38	44	12.66	36	6.82	45	0.434	y	2.39E-09	(+)	+	+	IQ>KI>PA
PCB136	42.14	51	31.45	38	21.74	46	0.373	y	8.09E-09	(-)	+	+	KI>IQ>PA
o,p'-DDD	202.62	47	247.80	47	196.99	48	0.373	y	9.74E-09	(+)	(+)	+	IQ>KI>PA
o,p'-DDT	1379.95	44	1572.48	43	819.49	41	0.452	y	9.96E-09	(+)	+	+	IQ>KI>PA
β-HCH	74.42	40	62.67	34	43.01	39	0.387		1.32E-08	(-)	+	+	KI>IQ>PA

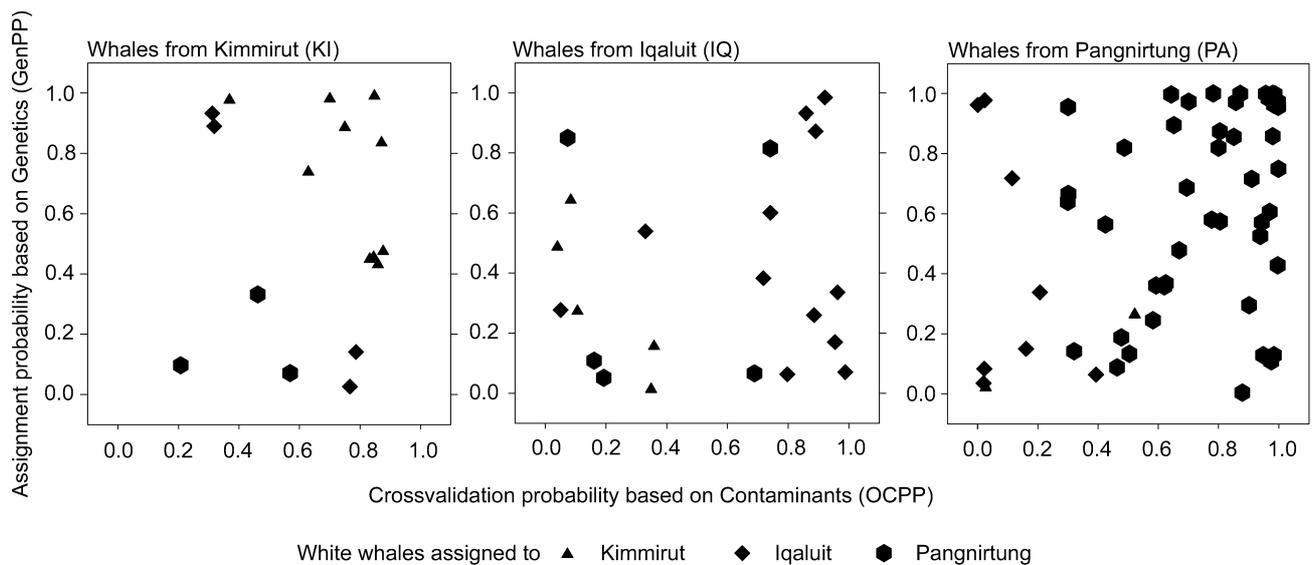


Fig. 2. Probabilities of assignment to three communities based on the Genetic Study and the OC study. The community to which each whale was assigned, based on the crossvalidation probability obtained from CDA combining the results of the two studies is given in the key.

effects. There were significant differences ($p \leq 0.0004$) among all three locations for the following 10 OCs: PCB42; PCB87; PCB153; PCB132; PCB105; PCB138; PCB187; PCB201/157; PCB180; and PCB196/203. Also, at $p \leq 0.0004$, concentrations of 34% (30/88) of OCs differed

significantly between PA and KI, 39% (34/88) between PA and IQ and 16% (14/88) between KI and IQ. The most common trend in mean OC concentrations was $IQ \geq KI > PA$ (Table 2). Covariates were not significant at $p \leq 0.05$ for three OCs that had location effects significant at $p \leq 0.0004$:

Table 3
Placement patterns and assignment probabilities (in brackets) in genetics, OC and joint studies.
PA = Pangnirtung, IQ = Iqaluit and KI = Kimmirut.

Column	Genetics		OC		Genetics		OC		Joint		
	All data		All data		Whales used in both studies						
	(1)	(2)	(3)	(3b)	(4)	(4b)	(5)	(5b)			
Direction of placement											
PA to PA	74	61%	46	73%	36	(0.846)	42	(0.822)	47	(0.922)	82.5%
PA to IQ	26	21%	10	16%	15	(0.672)	6	(0.795)	8	(0.850)	14.0%
PA to KI	22	18%	7	11%	6	(0.651)	9	(0.628)	2	(0.785)	3.5%
Total <i>n</i> PA	122	100%	63	100%	57		57		57		100%
IQ to PA	15	18%	3	13%	6	(0.636)	3	(0.662)	5	(0.781)	21.7%
IQ to IQ	42	51%	13	54%	10	(0.721)	13	(0.850)	13	(0.848)	56.5%
IQ to KI	26	31%	8	33%	7	(0.718)	7	(0.739)	5	(0.847)	21.7%
Total <i>n</i> IQ	83	100%	24	100%	23		23		23		100%
KI to PA	14	22%	3	8%	1	(0.622)	0		3	(0.748)	17.6%
KI to IQ	20	31%	10	27%	5	(0.727)	4	(0.535)	4	(0.772)	23.5%
KI to KI	31	48%	24	65%	11	(0.762)	13	(0.753)	10	(0.869)	58.8%
Total <i>n</i> KI	65	100%	37	100%	17		17		17		100%
Total <i>n</i>	270		124		97		97		97		

endosulfan, β -HCH and mirex. There were significant covariate effects but not significant location effects in 10 OCs: dieldrin; PCB17; PCB31; PCB52; PCB91; PCB83; PCB179; PCB200; o,p'-DDD; and Σ CHB ($p > 0.05$).

All 15 OC groups had location effects significant at $p \leq 0.05$, however only seven groups were significant at $p \leq 0.006$, the minimum significance level for table-wide comparisons among 15 groups (Table 2). The probability of location effects was highest for $\Sigma 7$ -CB ($p = 1.77 \times 10^{-10}$) and lowest for $\Sigma 3$ -CB ($p = 0.0356$).

In the CDA, the first discriminant function with the described 13 predictor variables accounted for 77% of the variance (Fig. 3). The first function mainly separated white whales from PA from the other two locations. The scores for the function were significantly correlated with 99 of 103 possible predictor variables ($p \leq 0.05$, 88 OCs and 15 OC groups in Table 2), most strongly with $\Sigma 7$ -CB ($r = 0.764$), PCB185 ($r = 0.754$), PCB187 ($r = 0.740$), PCB180 ($r = 0.719$), mirex ($r = 0.712$), all $IQ > KI > PA$ and PCB193 ($r = 0.748$, $KI = IQ > PA$). The second canonical function mainly separated white whales from KI and those from IQ (Fig. 3). The scores were significantly correlated with 55 of 103 predictor variables, the highest correlations with octachlorostyrene ($r = 0.626$, $IQ > PA > KI$), PCB194 ($r = 0.512$) and PCB199 ($r = 0.437$), both $IQ > KI = PA$, C3 (a chlordane isomer) ($r = -0.410$, $KI \geq IQ \geq PA$), and PCB105 ($r = 0.406$, $IQ > KI > PA$). Mean concentrations of OCs were significantly ($p \leq 0.05$) higher in IQ than in KI in 16 OCs and 3 OC groups ($\Sigma 6$ -CB, $\Sigma 7$ -CB, $\Sigma 8$ -CB). Mean OC concentrations were significantly higher in KI than in IQ in only 3 OCs: PCB05 (2-CB), PCB45 (4-CB), γ -HCH and in none of the OC groups (Table 2).

In stepwise CDA of the same 13 predictor variables, the following were chosen in order at $p \leq 0.05$: $\Sigma 7$ -CB; Σ DDD; octachlorostyrene; mirex; endosulfan; Σ CHL and $\Sigma 6$ -CB; and the remaining 6 OCs were not significant. The first 7 OC groups chosen had slightly different patterns of significant differences from each other among communities (Table 2). Pairwise plots of the OCs that were chosen by the stepwise CDA suggests that the major OC differences among communities may be best described as OC ratios, for example the Σ DDD/ $\Sigma 7$ -CB ratio (Fig. 4). In stepwise CDA

of all 88 predictor variables, PCB187, o,p'-DDD and PCB136 were the first three predictor variables chosen, suggesting similar ratios.

Of 124 whales in the OC study, 67% (83/124) were correctly crossvalidated to their source hunting community (46/63=73% from PA, 13/24=54% IQ and 24/37=65% KI) (Table 3). As in the genetics study, whales from KI and IQ were often misassigned to each other (33% and 27%), while misassignment percentages between whales from PA and whales from IQ or KI were lower, ranging from 8-16%. Individuals that were misassigned had intermediate canonical scores (Fig. 3). Patterns of assignments and misassignments were not significantly different between the 124 whales and the subset of 97 used in both studies (comparisons of columns 2 and 4 in Table 3, Chi Squared = 7.148, $p = 0.5207$, 8 df).

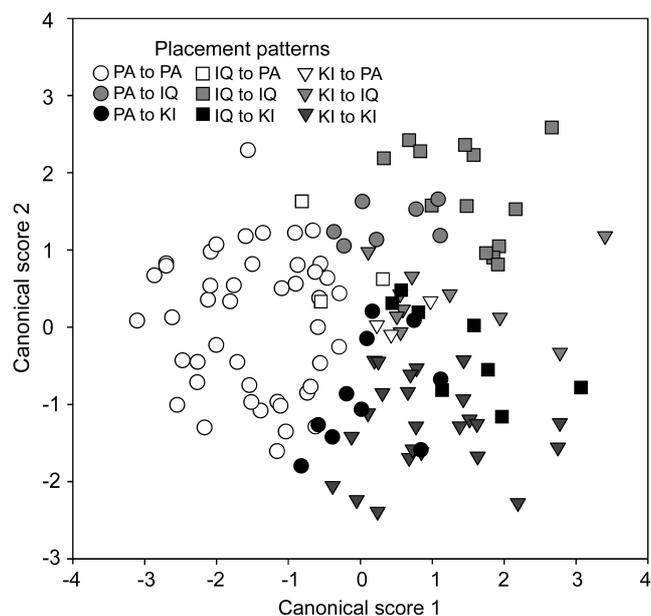


Fig. 3. Canonical Score 1 \times Canonical Score 2 from CDA of OC data from 124 white whales. The key identifies both the source hunting community and the community to which the individual was assigned.

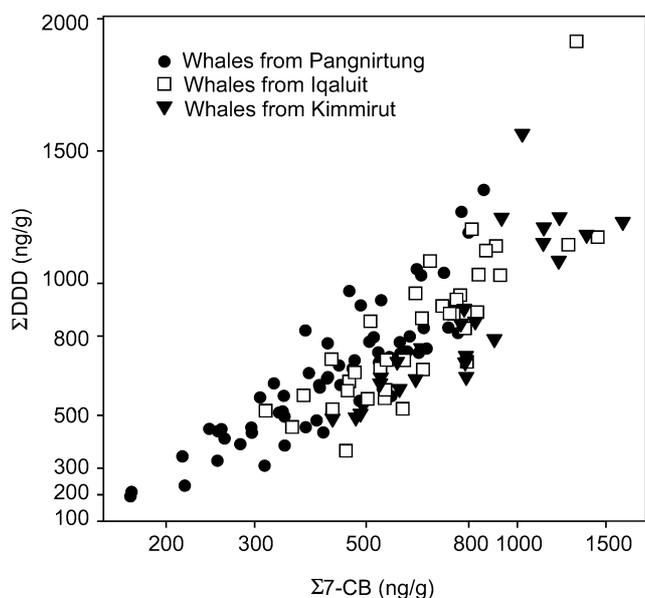


Fig. 4. Σ DDD versus Σ 7-CB (ng/g) in 124 white whales.

Joint study

Comparisons of assignments

Mean probabilities of assignment to the source communities in the OC study were similar to those in the genetics study (columns 3a versus 3b, Table 3). In general, a few more individuals were placed to their source hunting community in the OC study, with the difference slightly stronger when the whole sample populations rather than the sub-sample of 97 individuals was considered (percentages from columns 1-4, Table 3). However, assignment percentages to the source hunting community were not significantly different between the two studies (Chi-square tests comparing columns 1-4, Table 3).

Nevertheless, individuals' assignments were not the same in both studies. The GenPPs and the OCPPs to the source hunting community were correlated for white whales from PA ($r=0.347$, $n=57$, $p=0.0083$), and not for whales from KI or IQ ($r<0.12$ and $p>0.6$, both locations) (slopes, Fig. 2). Fisher's Exact Test (Kendall and Stuart, 1967, pp.580-585) applied to whales from each source, comparing assignment of individuals to 3 source communities in the OC study \times 3 source communities in the genetics study, elucidated details of assignment differences. Patterns of individual assignment in the two studies were independent for whales from both IQ and KI ($p=1.00$, both cases). Assignment patterns were not independent for whales from PA ($p=0.0465$). In this comparison, there was more than an expected number of PA whales placed to PA on the basis of both genetics and OCs.

Joint assignments in genetics and OC studies combined

The first discriminant function using assignment probabilities as predictor variables in the CDA in the Joint Study accounted for 80.7% of the variance. The first score was strongly correlated with the OCPP for PA, and weakly with OCPP for IQ and GenPP for KI ($p\leq 0.05$). The second score was significantly correlated with the OCPP and squared values of the OCPP for all three communities.

In crossvalidations using JointPPs, 46 of 57 (80.7%) white whales from PA, 14/23 (60.9%) from IQ and 11/17 (64.7%) from KI were assigned to their source hunting community (Fig. 2; column 5, Table 3). As in both the genetics and OC studies, the highest percentages of

misassignments were between whales from KI and IQ (23.5 and 30.4%), and percentages of misassignments between PA and the other communities were lower, between 3.5% and 15.8%. Assignment patterns determined from JointPPs did not differ significantly from assignment probabilities in either component study (column 5 versus columns 1 to 4, Table 3, Fisher's Exact Test, $p>0.05$ all cases).

The crossvalidation probabilities from this CDA were slightly larger than those in component studies (Fig. 5, columns 6 and 7 versus 8, Table 3). Groups of assigned and misassigned whales could not be related to sampling years, season, sex or age.

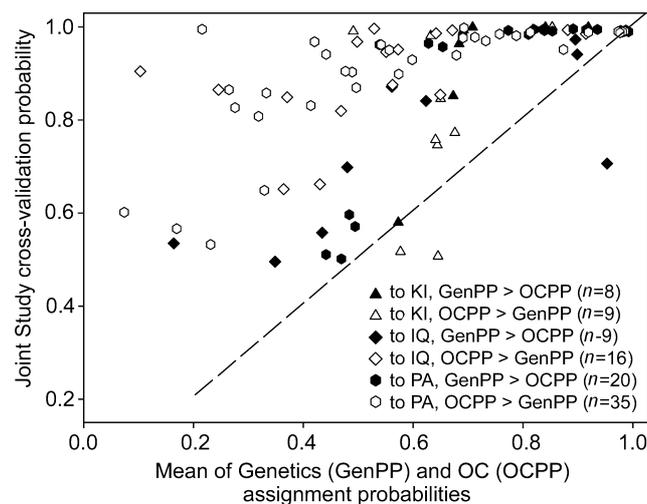


Fig. 5. Assignment probabilities in the Joint Study versus mean of assignment probabilities to the same communities in the Genetics and OC studies. The key also describes which one of the two probabilities used to calculate the mean was larger.

DISCUSSION

Component studies

The assignment probabilities and percentages of misassignments calculated from genetics data among whales from the three communities are weakly correlated with genetic distances demonstrated in the previous study of de March *et al.* (2002). The highest percentages of misassignment were between KI and IQ, which had the smallest genetic distances between them, while the lowest percentages of misassignments were between KI and PA, which had the largest. In addition, rates of assignment to the source community were related to the presence of uncommon haplotypes, this in turn related to the genetic diversity. Whales from PA, which had a high diversity due to several unique and uncommon haplotypes, were most identifiable. Whales from KI had a low haplotype diversity, but had two haplotypes otherwise associated only with Foxe Basin samples (de March and Postma, 2003).

OC concentration values are similar to those previously observed in white whales from the Arctic (Muir *et al.*, 1992; Stern *et al.*, 1994; de March *et al.*, 1998; Krahn *et al.*, 1999). Results described here also support the patterns described by Innes *et al.* (2002) in that concentrations of most OCs were higher in KI than in PA. The patterns in covariate coefficients show that there is a higher rate of OC uptake in adult males than in females for most of the OCs examined, as expected, since females transfer OCs to their young.

Univariate and multivariate differences among concentrations of OCs and OC groups in whale whales hunted in KI, IQ and PA, support the hypothesis that some whales hunted in the three SEB communities are from different stocks. The first canonical function in the analysis of OC data strongly separated whales hunted in PA from those hunted in IQ and KI. It is possible that the Cumberland Sound food web is 'cleaner' than that of whales hunted from the other two communities. The second discriminant function was dominated by OCs of which concentrations in whales from IQ were significantly different than those from KI, most often $IQ > KI$. Particularly, OCs that are characteristic of animals that feed higher in the food chain, namely 6-, 7- and 8- chlorinated PCBs had higher mean concentrations in whales from IQ. It is possible that whales from IQ feed in an 'Atlantic' food web that differs from the Cumberland Sound and Hudson Bay food webs. There are no reasons to believe that the sources of OCs for whales from IQ are local. A small number of OCs, namely PCB05 (2-CB), PCB45 (4-CB), γ -HCH, Σ CHB, Σ HCH and Σ CHL had higher concentrations in KI whales than in IQ whales, and even lower concentrations in PA whales. Some of these OCs have lower fat solubilities than the higher chlorinated PCBs ($K_{ow,s} = 3$ to 4 versus $K_{ow,s} > 6$), and are metabolised faster. Most of these whales from KI were hunted in the autumn; thus this difference may reflect unknown aspects of recent feeding of KI whales, presumably in the summering areas in Hudson Bay or Foxe Basin.

OC differences among whales hunted from the three communities can be interpreted as differences in OC ratios as well as in concentrations. This manuscript demonstrated the Σ DDD/ Σ 7-CB ratio (Fig. 4). DDD is a microbial degradation product most often found in sediments, and Σ 7-CB indicate a high food web level. The ratio is highest in whales from PA than in whales from KI and IQ, suggesting that whales from PA are feeding lower in the food web, perhaps on benthic fish or fish that feed on benthos. The ratio is smallest for whales from KI even though concentrations of both Σ DDD and Σ 7-CB are notably higher in KI than in PA. In view of the poor knowledge about feeding in these whales, it is difficult to interpret these ratios.

Combined results

Analysis of genetics and OC data jointly gave slightly stronger evidence for stock differences than in the component studies because some whales, particularly those from PA, had both OC and genetics signatures that associated them with the same hunting community. The degree of stock discrimination was only slightly more convincing in the joint analysis than in the component analyses. The CDA showed that OC data were more important for describing stock differences than the genetics data. Whales hunted in PA and IQ are identifiable mainly by their contaminant signature, although a notable fraction of whales from PA also had a strong PA genetics signature. Approximately half of whales hunted in KI had both a strong OC and genetics signature for KI.

In general, patterns of assignments among whales hunted in SEB communities suggest the possibility that the stock of whales hunted in KI and IQ might also be hunted in PA, but that a stock hunted in PA is not hunted in the other two communities. These results are consistent with the hunters' belief that more than one group of whales comes into Cumberland Sound (Kilabuk, 1998). In this study, 8 of 10 misassigned whales from PA were placed to IQ, with an

average assignment probability 0.850. Cumberland Sound is a considerably more productive area than Frobisher Bay and it would be attractive to white whales.

Hunters in Kimmirut also believe they hunt two groups of whales. Of 35 whales hunted in KI for which dates were available, 11 were hunted before August and 24 after August. However, whales hunted in the two seasons showed no differences in assignment patterns.

Whales from KI and IQ are discriminated from each other in the Joint Study to the same extent as in the component studies. In view of this result, it must be considered that most individuals from KI and IQ are members of stocks subject to gradients. It is also possible that more than two stocks are hunted in these two communities, and this blurred results in this study. The similarities between these two hunted groups are difficult to explain in view of the fact that the summering locations are widely separated. A partial explanation may be that wintering areas and mating areas overlap. If this were true, it would still be possible to observe haplotype differences between the two areas, and this was not the case. Another explanation may be that the stocks of whales hunted in KI and IQ do not utilise the same summering areas their entire life, or that summering areas will change with time or with generations. It has even been suggested that some whales travelling toward Hudson Strait in the spring are diverted north by the land they encounter (P. Richard, pers. comm.). These whales then enter Frobisher Bay and some possibly parts of Cumberland Sound.

In conclusion, we believe that members of at least three different stocks are sampled in the three SEB communities. However, these stocks may overlap in geographic ranges, and the stock hunted in KI and IQ have similar genetic characteristics. These results support management decisions that have been made.

Statistical considerations in combining results of different types of studies

When the results of different studies using the same subjects are combined, a measure of the covariance between responses in the two studies is desirable to reduce or quantify the residual error. If this covariance cannot be estimated, final conclusions can be based only on averages or on results weighted by the perceived importance of component studies. For this reason, multivariate statistics using results from all component studies, which take into account the covariance among responses from both studies, are a desirable method for analysing results.

'Responses' measured for the same test subjects in component studies can consist of raw data (OC concentrations, allelic information) to responses derived from the raw data (scores, probabilities, distances). A multivariate analysis which combines both OC and genetics raw data would require an underlying model with both continuous variables for the OC data and nested class variables (for alleles within loci) for the genetics data. Desirable output would consist of the comparison of linear combinations of OC data and also comparisons of variance components for the genetics data. At the present time, software for such an analysis is not available as a unit. Even if such an analysis were carried out, a model with many predictor variables might be overparameterised (see below). Thus the decision here to use summary responses from individual studies, was attractive in view of computationally difficult alternatives.

Before a researcher can consider combining the results, options in analysis of component studies must first be addressed. Studies with relatively few animals and many measurements, as both component studies are in this case, can be analysed by a diversity of numerical techniques which have different underlying hypotheses and which are known to have different biases. These biases can interact in a combined analysis. For example, if methods for describing both types of data are biased toward individuals' resembling other individuals from their source population, then the results of the different studies are more likely to be similar. For example, if whales from PA that had unique alleles also had a strong PA contaminants profile, we could be more confident in concluding that these alleles characterise whales from PA. Thus, the use of several types of data for scoring the same individuals can form a feedback loop in which the results of one study lead to that evaluation of numerical methods in the other. Of course, this feedback process is more likely to occur if some true stock differences exist and some predictor variables are both highly relevant and precise.

With respect to appropriate predictor variables in studies of OC contaminants, the use of many OCs must be carefully considered. Although a large number of OCs can be measured with a high degree of accuracy, the use of multivariate statistics and a large number of variables can yield results which are not representative of true differences among sampled populations ('overparameterised models' in Tabachnick and Fidell, 2001). Overparameterisation may be first noticed in multivariate data analyses when an unexpectedly high degree of discrimination among populations is obtained. This overparameterisation seems to disappear when individuals are crossvalidated (Lachenbruch and Mickey, 1968). However, in spite of the more plausible result after crossvalidation, Tabachnick and Fidell (2001) believe there may still be a lack of power. In other words, the number of individuals that are crossvalidated to their true source may not have improved. To avoid this, Tabachnick and Fidell (2001) recommend that the sample size of the smallest test group should 'notably' exceed the number of predictor variables. This is one reason why OC groups rather than individual OCs were used in these analyses.

Predictor variables can also be chosen to optimise discrimination among populations. Although 'stepwise selection' techniques can be informative for exploratory work, chosen predictor variables may describe differences that do not reflect true population differences (Tabachnick and Fidell, 2001). If there are many predictor variables, and particularly if experimental errors are associated with them, stepwise selection methods will find a combination of predictors that discriminate populations 'too' well.

With respect to molecular genetics studies similar problems can occur if AMOVA (Analysis of Molecular Variance; Excoffier *et al.*, 1992) and other common techniques for genetic analyses are used to analyse many loci, especially if some are 'meaningless'. Allele frequencies may have been used in an appropriate canonical CDA for the joint study with a nested structure for alleles within loci; however, this would have added 16 more predictor variables (loci) to the analysis.

For the genetics study, we chose to use assignment probabilities as summary statistics and predictor variables for the Joint CDA. One of the most important considerations in using assignments is that they are responsive to rare and uncommon alleles. We substituted 0.5 for all '0' allele frequencies within populations before assigning individuals,

as suggested by Waser and Strobeck (1998). If this substituted frequency is set to '0' or a smaller value, then more or all individuals with unique or rare alleles cannot be assigned, or are assigned to their source hunting population because of the unique allele. If this value is higher, then more individuals would be assigned to populations with common alleles. The analysis is thus also dependent of sample size, since a rare allele will have a higher frequency in a small population than in a large one. Thus different choices in the assignment methods would have yielded slightly different assignments in the genetics study, and this would have been passed into the larger analysis.

In conclusion, other summary statistics for component studies and therefore predictor variables for the joint study could have been chosen. The balance between choosing a predictor variable that might discriminate 'too' well due to chance and predictor variables that blur results because they are meaningless must be considered.

Assignment probabilities are one method of scoring or describing individuals. However, actual assignments cannot be considered to represent degrees of mixing. All 'assignment' methods, based on genetics or OC data, do not take into account that: (1) different distinct stocks should be at Hardy-Weinberg (breeding) equilibrium, thus some genotypes in each stock will resemble individuals in another stock more than in their own; and (2) if there are normal distributions of OC concentrations within each stock, then some individuals in each stock will resemble individuals in another stock more than in their own. Because of this, some individuals will be assigned to hunting sources other than the source community. Future methods, in genetics studies, OC studies, and combined studies, should concentrate on separating overlapping similarity distributions.

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