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I. Bakke · S. Johansen · Ø. Bakke · M. R. El-Gewely

Lack of population subdivision among the minke whales (*Balaenoptera acutorostrata*) from Icelandic and Norwegian waters based on mitochondrial DNA sequences

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Abstract The minke whale (*Balaenoptera acutorostrata*) is subject to commercial whaling, but stock identification and assessment are still uncertain. Mitochondrial DNA (mtDNA) sequences were determined to examine the population structure of minke whales from the central and northeastern parts of the North Atlantic, as well as the Antarctic regions IV and V. The analyses include 345 nucleotide positions of the control region of 110 individuals, and 250 nucleotide positions of the NADH dehydrogenase subunit 2 gene for a representative selection of North Atlantic minke whales. Maximum parsimony analyses and sequence divergence calculations did not reveal any genetic differentiation between individuals from the central and northeastern parts of the North Atlantic. These results do not support the International Whaling Commission's separation of minke whales in this area into different management units, and they are in conflict with previously reported results from allozyme analyses. Comparison of minke whale control region sequences showed that the sequence diversity of North Atlantic minke whales is substantially lower (0.0065) than that of Antarctic minke whales (0.0166), and clearly demonstrated that individuals from these two areas represent genetically distinct populations.

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I. Bakke (✉) · M.R. El-Gewely
Department of Biotechnology, Institute of Medical Biology, University of Tromsø, N-9037 Tromsø, Norway

S. Johansen
Department of Molecular Cell Biology, Institute of Medical Biology, University of Tromsø, N-9037 Tromsø, Norway

Ø. Bakke
Norwegian Institute of Nature Research, Tungasletta 2, N-7005 Trondheim, Norway

Introduction

Among the approximately 76 living species of whales, the minke whale (*Balaenoptera acutorostrata*) is one of the very few that are still subject to commercial whaling. The hunting has led to scientific and political controversy (see Skåre 1994, and opposing comments therein), which in part is due to uncertainties in stock identification and assessment. Strong evidence is present that minke whales of the North Atlantic, North Pacific and Antarctic ocean basins represent distinct populations (Palsbøll 1989; Amos and Dover 1991; Wada and Numachi 1991; Wada et al. 1991; Árnason et al. 1993; Hori et al. 1994; van Piljen et al. 1995). It has been suggested that three different subspecies of minke whale exist: *B. acutorostrata acutorostrata* (North Atlantic), *B. acutorostrata davidsoni* (North Pacific) and *B. acutorostrata bonaerensis* (Antarctic) (reviewed in Horwood 1990). The subspecies *B.a. davidsoni* has been questioned (see Horwood 1990), but several studies conclude that *B.a. bonaerensis* should be given full species status (Omura 1975; Arnold et al. 1987; Wada and Numachi 1991; Árnason et al. 1993). The population structures within these oceans are less well known. Based on catch distributions, segregation by sex and length, sightings, and marking data, the International Whaling Commission (IWC) divided the minke whales of the North Atlantic into four management units; the Canadian east coast, the West Greenland area, the central area, and the northeastern area (Fig. 1A). The population structure of the North Atlantic minke whale has been investigated by several techniques, such as allozyme loci variability (Danielsdottir et al. 1992), DNA fingerprinting (Árnason and Spillaert 1990), restriction fragment length polymorphism of mitochondrial DNA (mtDNA) (Palsbøll 1989), and morphometric comparisons (Christensen et al. 1990). While the two former studies found evidence for some genetic differentiation between the West Greenland, the central and the northeastern area, no certain conclusions about the

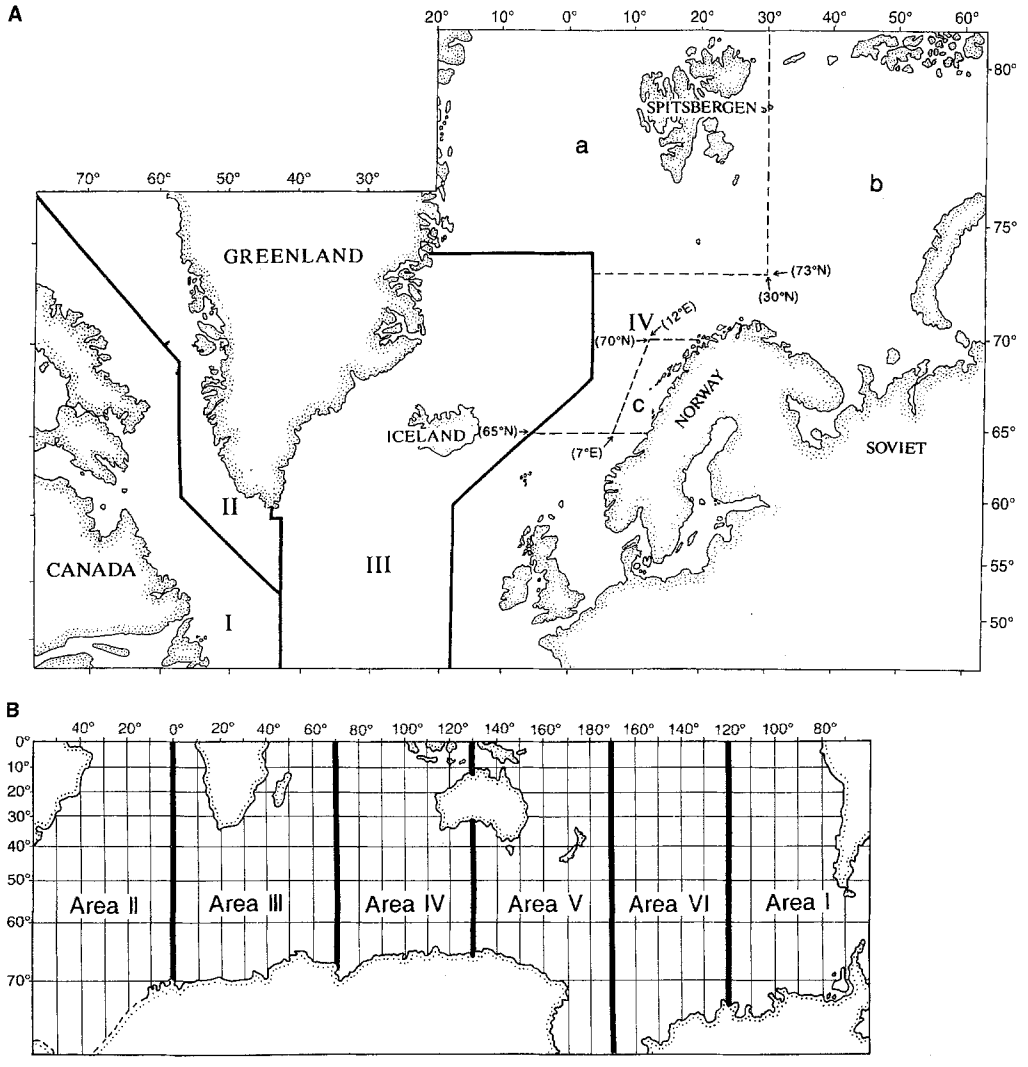


Fig. 1 IWC management regions for **A** North Atlantic **B** and Antarctic minke whales. *Dashed lines* give boundaries for the IWC's "small areas" of the North Atlantic that are considered in this study. [*I* Canadian east coast; *II* West Greenland; *III* central area; *IV* northeastern area (*a*: ES; *b*: EB; *c*: EC)]

existence of isolated breeding populations could be drawn from the morphometric comparisons. The restriction analysis of mtDNA did not show any genetic differentiation between the different areas. Whether the management units of North Atlantic minke whales represent different populations based on genetic and/or breeding characteristics, is still questionable.

Due to its simple structure and evolution, mitochondrial DNA has been used as a marker to assess genetic variability among animals at different taxonomic levels (Meyer et al. 1990; Kraus and Miyamoto 1991; Gatesy et al. 1992; Milinkovich et al. 1994; Taberlet and Bouvet 1994). Application of mtDNA analysis as a tool in conservation biology is well established (see Moritz 1994), and analysis of mtDNA variation has been performed for a number of cetaceans (Baker et al. 1990; Hoelzel and Dover 1991; Schaeff et al. 1991; Wada et al. 1991; Baker et al. 1994; Rosel et al. 1994). Complete mtDNA sequences of the fin whale and blue whale (Árnason et al. 1991; Árnason and Gullberg 1993), as well as the development of polymerase chain reaction (PCR) based direct sequencing (reviewed by Rao 1994), have encouraged analyses at the nucleotide sequence level. It is well known that different genes or regions in mtDNA of animals evolve at different rates (reviewed in Wolstenholme 1992). Portions of the non-coding control region have been shown to evolve rapidly in mammals (Cann and Wilson 1983; Brown et al. 1986; Saccone et al. 1991), making the region a useful marker to assess genetic variability and differentiation among populations or stocks. The control region has been sequenced from more than a dozen whale species (Árnason et al. 1993). Although Hoelzel et al. (1991) showed that the evolution rate of the control region is slower for cetaceans than for other mammals, this part of the mitochondrial genome has recently been successfully used in population studies of both humpback whales (Baker et al. 1993; Palsbøll et al. 1995) and

common dolphins (Rosel et al. 1994). Thus, both interspecific and intraspecific applications of mitochondrial control region sequences have been exemplified in whales.

Here we report on a population study of a total of 110 minke whales from the central and northeastern parts of the North Atlantic, as well as the Antarctic areas IV and V (Fig. 1). We compared 345 nucleotide positions in a variable part of the mtDNA control region from all individuals and an additional 250 base pairs of the less variable mitochondrial NADH dehydrogenase subunit 2 (ND2) gene from 18 North Atlantic minke whales. The Antarctic and North Atlantic minke whales were found to be genetically distinct populations. In contrast, no significant population subdivision was observed among assayed North Atlantic minke whales.

Material and methods

Minke whale tissue samples and DNA extractions

Liver tissue was collected from a total of 110 captured individuals of the minke whale, *Balaenoptera acutorostrata*. A total of 46 samples were collected in the northeastern parts of the North Atlantic during the Norwegian scientific whaling expedition by the Department of Arctic Biology, University of Tromsø. Of these samples, 15 were collected in 1989 along the western coast of Spitsbergen ("small area" ES, Fig. 1A); 26 were collected in 1988 (23 and 3 from the small areas EC and EB, respectively) and 5 (3 and 2 from the "small areas" EC and EB, respectively) in 1990 along the coast of northern Norway. Samples from 41 individuals captured between 1981 and 1984 in the adjacent coastal waters west and north of Iceland (IWC central area) were supplied by A. Árnason (Marine Research Institute, Reykjavik). A total of 23 Antarctic samples were supplied by L.A. Pastene (Institute of Cetacean Research, Tokyo), which included 13 and 10 samples collected in the IWC management areas IV and V, respectively (see Fig. 1B). The Antarctic minke whales were captured between 1990 and 1992 by Japanese scientific whaling. Total DNA from liver tissue was extracted according to the procedure described by Kocher et al. (1989).

PCR amplification and DNA sequencing

The primers used for amplification of the mtDNA control region were complementary to sequences in the $tRNA^{Phe}$, $tRNA^{Pro}$ and $tRNA^{Thr}$ genes. The PCR primers are H598 [5'-A($A^{60}G^{40}$)I($G^{80}A^{20}$)CATTTTCAGTG($C^{60}T^{40}$)($C^{40}T^{40}A^{20}$)TTGCTTT-3'], L16005 [5'-($T^{60}C^{40}$)($C^{80}A^{20}$)A($A^{60}G^{40}$)C($A^{80}C^{20}$)CCCAAAGCT($G^{80}A^{20}$)($A^{80}G^{20}$)($T^{60}A^{40}$)($A^{80}G^{20}$)TTCT-3'] and L15926 [5'-TAGTATAAAIA($A^{60}T^{40}$)TACICIGGTCTTGTAAACC-3']. The ND2 amplification primers were located in the $tRNA^{Met}$ (L4428; 5'-GGTCAGCTAAITAAGCTATCGG-3') and $tRNA^{Trp}$ (H5534; 5-AGGGCTTTGAAGGCTCTTGG-3') genes. Several of the nucleotide positions in the primers are degenerated either as a variable site or by incorporation of inosine (I). Primers used for PCR and DNA sequencing are numbered relative to the position of the 3'nucleotide of the corresponding human sequence (Anderson et al. 1981). L and H refer to the light and heavy strands, respectively. PCR amplifications were performed using *Taq* polymerase as described by the supplier (Perkin Elmer Cetus Corporation). Biomagnetic Beads (Dynabeads M-280 Streptavidine; Dynal) were used for solid support of the amplified products. One of the primers used in the amplification reactions (H598 for the control region and H5534 for the ND2 gene) was biotinylated at the 5'end to allow binding of the PCR product to streptavidine-coated magnetic beads for direct solid-phase sequencing (Hultman et al. 1989). DNA sequencing reactions were performed using the T7 Sequencing Kit (Pharmacia) and [^{35}S]dATP (10 μCi μl^{-1}; New England Nuclear) as the label, as well as the primers L16005, L15926 or L4428.

Computer analysis of DNA sequence information

The software package program (Version 7) from Genetics Computer Group (Madison, Wisconsin) was used to enter and align sequences. The genetic relationship between genotypes was determined using the maximum parsimony (MP) method of the PHYLIP package (DNAPARS, Version 3.51c, Felsenstein 1993). MP trees were evaluated using the bootstrap procedure (Felsenstein 1985) based on 500 resamplings. The blue whale control region sequence (Árnason and Gullberg 1993) was used for rooting the tree.

Several calculations were made based on multialignment of the individuals' sequences. The average number of substitutions per nucleotide site (sequence divergence) between different genotypes was calculated by the method of Jukes and Cantor (1969). Nucleotide diversities within and between populations were calculated as described by Nei (1982). The interpopulational nucleotide diversity is given by $\delta_{ST} = \pi_T - \pi_S$, where π_T is the total nucleotide diversity disregarding population subdivision and π_S is the average nucleotide diversity within populations. The proportion of interpopulational nucleotide diversity is given by $\gamma_{ST} = \delta_{ST}/\pi_T$. The parameter γ_{ST} was chosen as an estimate of the extent of population differentiation since we make inferences about the sampled populations, not a larger collection of populations (Lynch and Crease 1990). The statistical significance of the genetic differences among individuals from different geographical areas was tested by performing a randomization test (Edgington 1987; Palumbi and Wilson 1990; Manly 1991; Palumbi et al. 1991). Individual genotypes were permuted among the different geographical areas, keeping the sample sizes the same as in the original analysis. After permuting, γ_{ST} was calculated. The permuting procedure was performed 999 times, giving 1000 values of γ_{ST} including the original γ_{ST}. Since under the null hypothesis (i.e. is no population subdivision) each possible rank from 1 to 1000 of the original γ_{ST} is equally probable, the achieved significance level of the test is the proportion of γ_{ST} values greater than or equal to the original γ_{ST}.

Results

Sequence variation and mtDNA genotypes

The 5'end of the mtDNA control region was chosen as a genetic marker to study the population structure and variability among minke whales. This region from 110 minke whales, representing both the North Atlantic and Antarctic branches, was amplified by PCR using the primer combination H598 and L15926. PCR products were then subjected to direct sequencing using the primer L16005 or L15926. Sequences representing the North Atlantic (N1) and the Antarctic (A1) stock of minke whales are presented in Fig. 2 and compared to the corresponding regions of the fin whale and blue whale. This region is located adjacent to the $tRNA^{Pro}$ gene and is known to be variable in sequence, but still homologous, between different whale species (Árnason

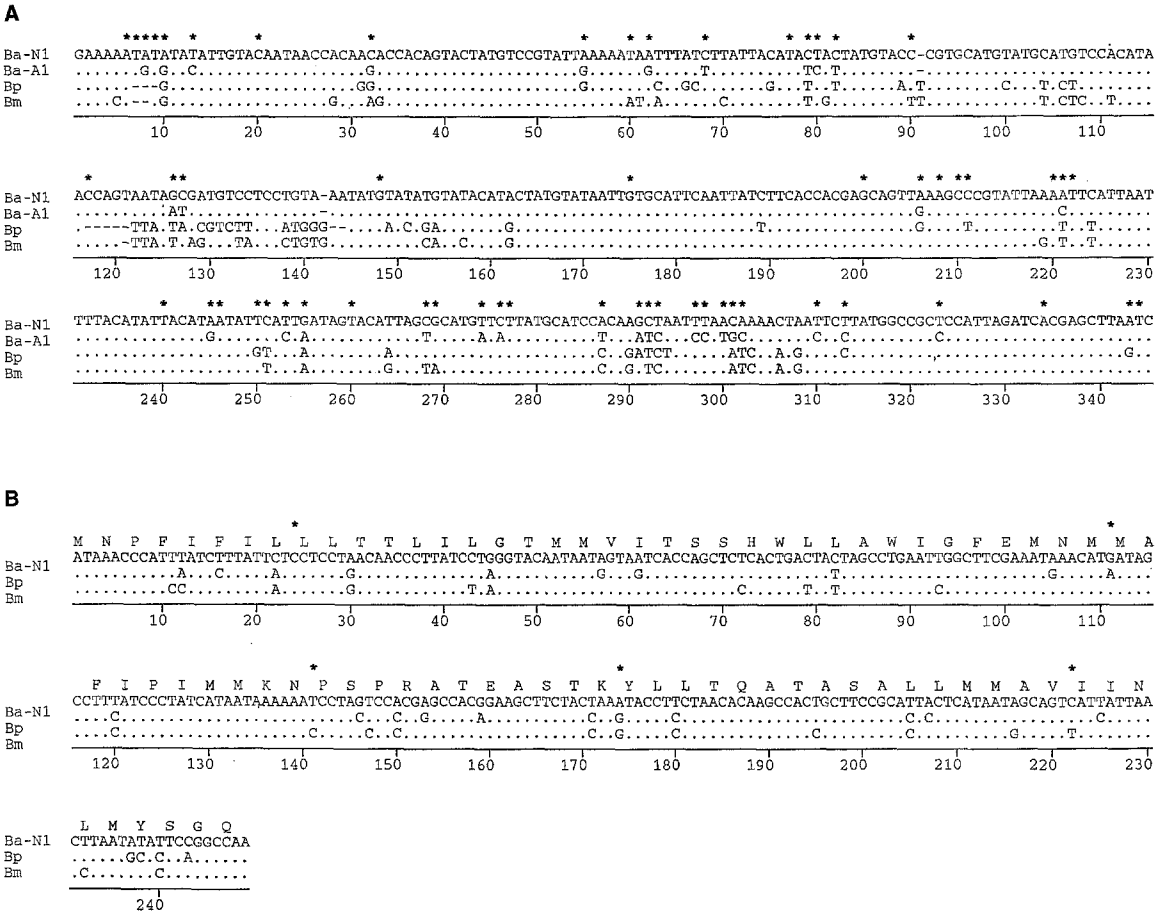


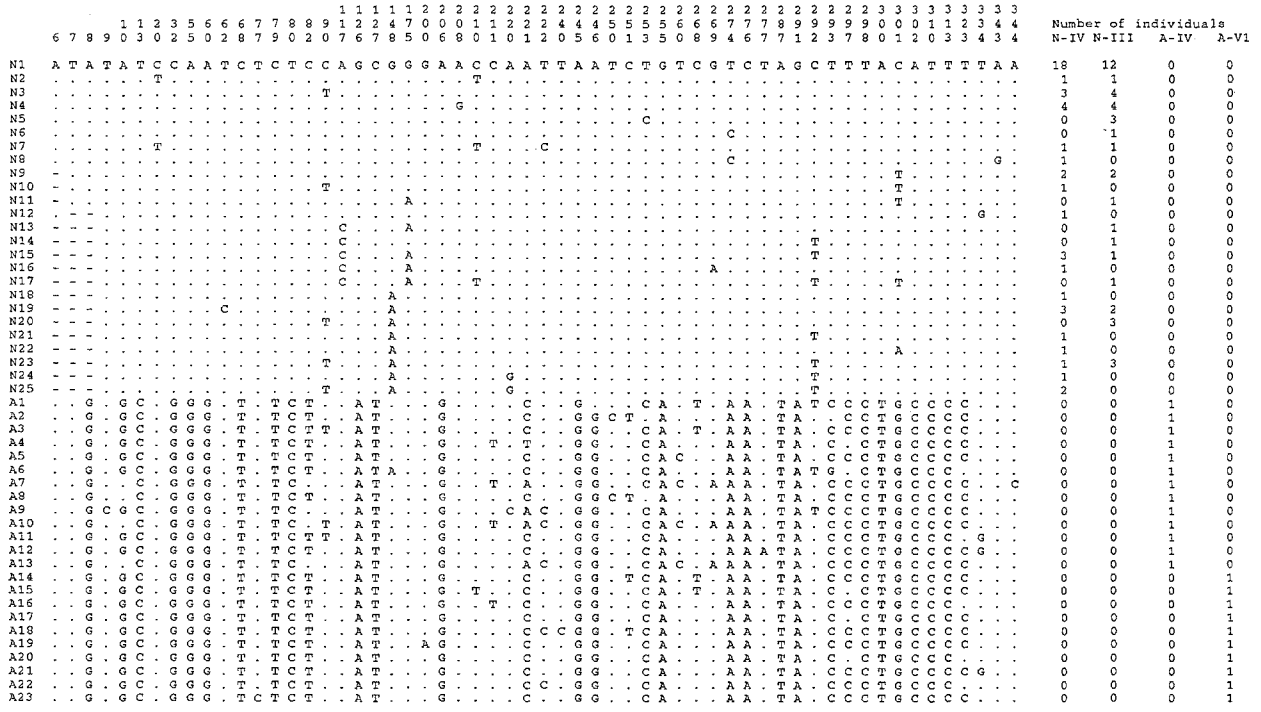
Fig. 2 *Balaenoptera* spp. North Atlantic and Antarctic minke whale mtDNA sequences of **A** the 5'-end of the control region and **B** the 5'-end of the ND2-gene. Minke whale sequences are aligned to corresponding fin and blue whale sequences (Árnason et al. 1991; Árnason and Gullberg 1993). *Asterisks* above the sequences indicate the variable positions among the genotypes determined (see Fig. 3). Identical positions are indicated by *dots* and deletions by *dashes*. [*Ba*-N1: *B. acutorostrata* genotype N1 (North Atlantic); *Ba*-A1: *B. acutorostrata* genotype A1 (Antarctic); *Bp*: *B. physalus* (fin whale); *Bm*: *B. musculus* (blue whale)]. The control region sequences *Ba*-N1 and *Ba*-A1 (**A**), and the ND2 sequence *Ba*-N1 (**B**) have been assigned the EMBL Data Library accession numbers X87773, X87774, and X87775, respectively

et al. 1993; Dillon and Wright 1993). The corresponding Antarctic minke whale sequence published by Hoelzel et al. (1991) differs by several insertions and deletions from the Antarctic minke whale sequences presented here. A total of 58 variable nucleotide positions were found among the assayed minke whales, and within the North Atlantic (N) samples 25 distinct genotypes (N1 to N25) were identified (Fig. 3A). The N-genotypes were defined by 20 variable positions, and the most common genotype (N1) was found in 30 individuals. Most genotypes are shared between individuals from different geographic areas, and the distribution of genotypes shows no apparent geographic structure. All the Antarctic individuals represent distinct genotypes. Fig. 3 summarizes the results from the DNA sequencing analysis and the distribution of genotypes in relation to the different geographical areas.

Maximum parsimony analysis

To investigate the relationship between the different genotypes a MP-analysis was carried out. A strict consensus tree based on 500 bootstrap replications is shown in Fig. 4. Confirmed by 100% of the replications, all the minke whale genotypes were distinct from the fin whale and blue whale sequences. Furthermore, the division of minke whale into two subgroups represented by the North Atlantic (N) and Antarctic (A) branches, and a further subdivision of the A-branch into two genotype groups was supported by 100% of the replications. A significant genotype structuring was not found within the North Atlantic branch. However, a clustering of N genotypes, though statistically weaker, is suggested by the MP-analysis. All the clusters include genotypes found in all localities, and no geographic clustering of the genotypes is revealed. To further examine if these clusters represent true genealogical branches, we analyzed 250 additional nucleotide positions (Fig. 2B) of the more conserved mt ND2 gene from 18 individuals, representing different clusters of

A



B

	2 4	1 2 1 1	1 4 7 4	2 2	No.of individuals	control region genotypes
ND2-1	C	G	T	A	C	9	N1, N5, N6
ND2-2	.	A	C	.	T	5	N20, N23, N24, N25
ND2-3	T	A	C	.	T	1	N20
ND2-4	.	.	C	G	T	3	N13, N15, N162

Fig. 3 *Balaenoptera* spp. Variable nucleotide positions in **A** control region and **B** ND2-gene of minke whale mtDNA. Positions of the variable sites according to sequences in Fig. 2 are shown vertically above reference sequence. Identity to reference sequence N1 is indicated with *dots*. *Dashes* indicate deletions. (*N-IV* northeastern North Atlantic area; *N-III* central North Atlantic area; *A-IV* Antarctic Area IV; *A-V* Antarctic Area V. North Atlantic and Antarctic genotypes are denoted *N* and *A*, respectively, in the left-hand column)

the North Atlantic branch on the MP-tree. Control region genotypes for these individuals are given in Fig. 2B. ND2 sequence variation among the individuals confirmed the pattern of variation found from the control region sequences (Fig. 3B).

Nucleotide diversity and population subdivision

The nucleotide diversity within groups of minke whales are given in Table 1. The nucleotide diversity is substantially greater in the Antarctic (0.0159) than in the North Atlantic region (0.0064). The nucleotide diversity between individuals from the North Atlantic and Antarctic regions is found to be 0.045, while the corresponding values between Antarctic regions IV and V and between the northeastern and central North Atlantic are 0.0011 and 0.000034, respectively (Table 2). This indicates no population subdivision between the two North Atlantic regions. To further investigate the possibility of population substructuring in the geographic areas included in this study, we estimated the extent of population differentiation by calculation of γ_{ST}. The values of γ_{ST} between different geographic areas are given in Table 2. We found that the North Atlantic and the Antarctic minke whales represent different populations ($P = 0.001$), while no genetic differentiation could be detected between the northeast and the central areas ($P = 0.911$) or between the central, EC, and ES (see Fig. 1) areas ($P = 0.667$). The two Antarctic areas IV and V were genetically different at the 10% level of significance ($P = 0.078$).

Discussion

Population structure of minke whales in the northeastern and central North Atlantic management areas

A total of 25 different genotypes were found among the 87 North Atlantic minke whales. Most of these were shared by individuals from different geographic areas,

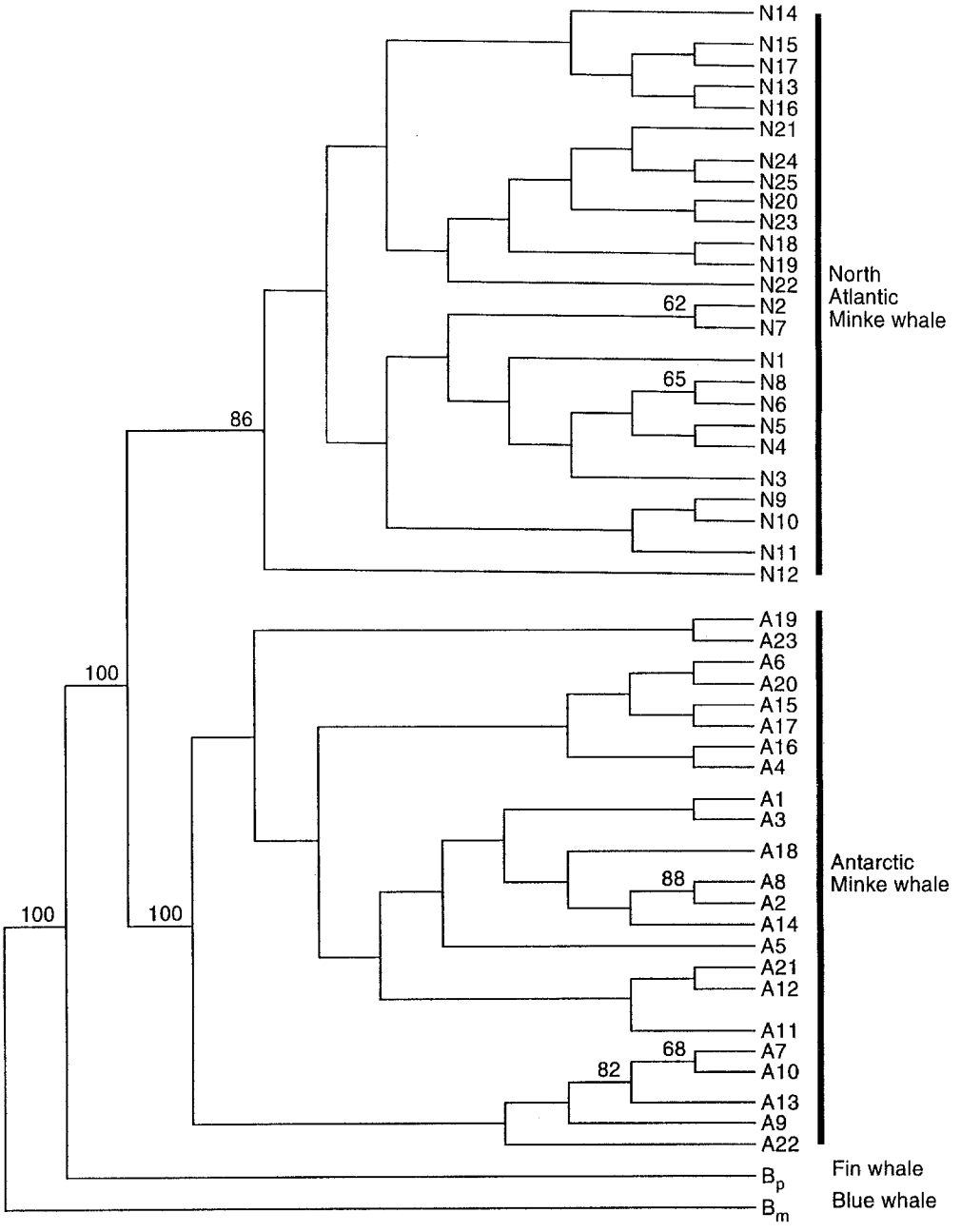


Fig. 4 *Balaenoptera* spp. A strict consensus MP-tree of minke whale control region genotypes and a fin whale (B_p) control region sequence, based on 500 replicates. Blue whale (B_m) control region sequence is used as an outgroup for rooting the tree. Bootstrap values are shown at nodes found in > 50% of the replicates; genotypes are defined in Fig. 3

and the distribution of the genotypes among the individuals seemed to be independent of geographic area. The MP-analysis did not reveal any population structure correlated to the different geographic areas. All clades in the North Atlantic branch include individuals from both the central and northeastern areas. The extent of population subdivision between different geographic areas was estimated by calculation of γ_{ST}. No support of population subdivision between central and northeastern areas or between the small areas EC and ES and the central area was found. The small area EB was not included because only five individuals, caught very close to area EC, represent this area.

These results are in conflict with earlier observations based on genetic variations at nuclear encoded enzyme loci (Danielsdottir et al. 1992) and hypervariable regions in the nuclear genome (Árnason and Spillaert 1990), which had led to the conclusion that the IWC management areas in the North Atlantic correspond to distinct minke whale populations. Morphological analysis was not conclusive, but suggested a geographic population structure (Christensen et al. 1990).

When the migration and breeding sex ratios are not biased, mtDNA usually shows more population subdivision than nuclear DNA (Birky et al. 1989). Due to maternal inheritance, mt genes have lower effective

Table 1 *Balaenoptera* spp. Nucleotide diversity within groups of individuals (π_i) from different geographical areas. (*NA* North Atlantic; *N-IV* northeastern North Atlantic area; *N-III* central North Atlantic area; *ES*, *EC* IWC small areas ES and EC; *Ant* Antarctic area; *A-IV*, *A-V* IWC Antarctic areas IV and V

Area	No. of individuals	π_i
NA	87	0.0064
N-IV	46	0.0064
N-III	41	0.0065
ES	15	0.0070
EC	26	0.0065
Ant	23	0.0159
A-IV	13	0.0193
A-V	10	0.0090

Table 2 Nucleotide diversity (δ_{ST}) and the proportion of interpopulational diversity (γ_{ST}) between minke whales from different geographic areas. *Abbreviations* given in Table 1

Areas	δ_{ST}	γ_{ST}	Significance level
NA/Ant	0.045	0.80	0.001
N-IV/N-III	0.000034	0.0052	0.911
A-IV/A-V	0.0011	0.070	0.078
N-III/ES/EC	0.00020	0.024	0.677

migration rates than nuclear genes (Birky et al. 1983). Random drift is faster for the haploid, maternally inherited mt genome compared to a diploid, biparentally inherited nuclear locus (Palumbi and Baker 1994). Furthermore, an accelerated substitution rate of the mitochondrial genome contributes to faster differentiation (Avise 1986). Examples from the literature include the North Pacific humpback whales (Baker et al. 1990; Palumbi and Baker 1994), marine turtles (Bowen et al. 1992), and island wolves (Wayne et al. 1991). In many cases, male-biased migration is a likely contributor to such patterns of differentiation.

A different pattern is observed among the North Atlantic minke whales. Here, nuclear encoded allozymes indicate more population subdivision than mtDNA sequences. In general, in certain situations with either the migration or breeding sex ratio biased strongly toward females, populations may show more subdivision for nuclear than mitochondrial genes (Birky et al. 1989). An alternative explanation could be non-representative evolutionary factors, e.g. selection on protein coding loci or differential expression of isoenzyme alleles among tissues. An example of the latter is the American oyster, where conflicting results from analyses of mtDNA and allozyme loci were found (Buroker 1983; Reeb and Avise 1990). Analysis of single copy nuclear DNA revealed that this discrepancy was probably due to balancing selection at allozyme loci (Karl and Avise 1992). Thus, to further evaluate the North Atlantic minke whale management units, mtDNA analysis should be used in concert with a comprehensive study of high-resolution nuclear markers.

The very few examples known where mtDNA show less geographic structuring than allozymes include the Red Hills salamander (McKnight et al. 1991) and Pacific populations of minke whales (Wada et al. 1991) [see discussion below of minke whale population structure in North Pacific as described by Goto and Pastene (1995)]. Moritz (1994) explains these observations by the fact that the mtDNA was uniform at the restriction sites examined, so that genetic drift accompanying recent separation of populations would not be apparent. However, this is not plausible for North Atlantic minke whales in which a number of mtDNA genotypes are found. As further pointed out by Moritz (1994), it will take thousands of generations for uniform but isolated populations to diverge through the accumulation of mutations (Palumbi et al. 1991), even when considering fast evolving sequences like the mtDNA control region. By contrast, changes in the frequency of mtDNA alleles can occur within an ecological time scale if the effective number of females is small. Moritz (1994) concludes that mtDNA provides a powerful tool for identifying management units within species, but only where multiple alleles have been identified.

When interpreting genetic data in population studies, factors like current and historical gene flow, migration pattern, and breeding structure should be considered. This kind of information is at present very limited for North Atlantic minke whale. Minke whales are widespread and seasonally abundant in the North Atlantic. Little is known about the breeding structure, except that breeding takes place in the winter in temperate waters. During spring and summer there is a general feeding migration northwards to Arctic waters (see Christensen et al. 1990). In the North Pacific, allozyme analyses indicated that different breeding populations overlapped at feeding grounds (Wada 1991). Recently, comprehensive analysis of fine-scale spatial and temporal distributions of minke whale mtDNA within the Antarctic (Pastene et al. 1995) and the western North Pacific (Goto and Pastene 1995) were reported. The analysis revealed complex population structures with overlap of different breeding populations and variable temporal patterns of distribution during the feeding season. A situation similar to this might exist in the North Atlantic. Temporal analysis was not performed in this study, but the small areas that were tested for population differentiation represented samples collected in different years. All the samples from the ES area (west of Spitsbergen) are from 1989, while all the samples from EC (the coast of northern Norway) except three are from 1988. The Icelandic samples were collected between 1981 and 1984. No population differentiation was found between these areas. However, temporal variation in the Antarctic (Pastene et al. 1995) and the North Pacific (Goto

and Pastene 1995) was detected after analyzing monthly variation in the feeding season. Two or more differentiated populations might co-exist at feeding grounds in the North Atlantic. This is a possible interpretation of the MP-tree in Fig. 4. The average nucleotide diversity (δ_{ST}) between the individuals in the two main North Atlantic clusters is found to be 0.0016, while the proportion of interpopulational nucleotide diversity (γ_{ST}) is 0.20 ($P = 0.001$). The possibility of co-existence of two breeding populations was first proposed by Palsbøll (1989). He found two main groups of genotypes when analyzing restriction fragment length polymorphism on mtDNA from North Atlantic minke whales. If there are differentiated populations of minke whales in the region considered in this study, they appear to be equally distributed in the three areas included in the statistical analysis. Temporal variation in the distribution of these potential populations can not be excluded.

Population differentiation between minke whales in the North Atlantic and Antarctic areas

The nucleotide diversity within the North Atlantic sample was found to be 0.0064, which corresponds to previously reported nucleotide diversity (0.0072) for North Atlantic minke whales (see Palsbøll et al. 1995). The nucleotide diversity within the Antarctic sample was found to be substantially larger (0.0159). This reflects a larger long-term effective population size of the Antarctic minke whale compared to the North Atlantic minke whale.

Both the MP-tree in Fig. 4 and the calculated population subdivision, γ_{ST}, strongly support the hypothesis that the North Atlantic and the Antarctic minke whales represent genetically isolated populations. This corroborates previous reports based on morphological, osteological and genetical comparisons of minke whales from the Northern and Southern Hemispheres which have advocated giving the Southern Hemisphere minke whale (*A. acutorostrata bonaerensis*) full species status (Omura 1975; Arnold et al. 1987; Wada et al. 1991; Árnason et al. 1993).

The minke whales from the Antarctic areas IV and V were found to be genetically different at the 7.8% level of significance. It is not possible either to exclude or conclude that there are different minke whale populations in these areas. A recent study by Pastene et al. (1995) indicated a complex nature of the population structure in these areas.

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References

Amos B, Dover GA (1991) The use of satellite DNA sequences in determining population differentiation in the minke whale. In: Hoelzel AR (ed) Genetic ecology of whales and dolphins. Report IWC, Special Issue 13, International Whaling Commission, Cambridge, pp 235–244

Anderson S, Bankier AT, Barell BG, deBruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature, Lond 290: 457–465

Árnason A, Spillaert R (1990) A study of variability in minke whale (*Balaenoptera acutorostrata*) in the North Atlantic using a human hypervariable region probe, alpha-globin 3'HVR. Paper SC/42/NHMi23 presented to the IWC Scientific Committee, June 1990

Árnason Ú, Gullberg A (1993) Comparison between the complete mtDNA sequences of the blue and the fin whale, two species that can hybridize in nature. J molec Evolut 37: 312–322

Árnason Ú, Gullberg A, Widegren B (1991) The complete nucleotide sequence of the mitochondrial DNA of the fin whale, *Balaenoptera physalus*. J molec Evolut 33: 556–568

Árnason Ú, Gullberg A, Widegren B (1993) Cetacean mitochondrial DNA control region: sequences of all extant baleen whale and two sperm whale species. Molec Biol Evolut 10: 960–970

Arnold P, Marsh H, Heinsohn G (1987) The occurrence of two forms of the minke whales in east Australian waters with a description of external characters and skeleton of the diminutive or dwarf form. Scient Rep Whales Res Inst, Tokyo 38: 1–46

Avise JC (1986) Mitochondrial DNA and the evolutionary genetics of higher animals. Phil Trans R Soc (Ser B) 312: 325–342

Baker CS, Palumbi SR, Lambertsen RH, Weinrich MT, Calambokidis J, O'Brien SJ (1990) Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales. Nature, Lond 344: 238–240

Baker CS, Perry A, Bannister JL, Weinrich MT, Abernethy RB, Clambokidis J, Lien J, Lambertsen RH, Urbán Ramírez J, Vasquez O, Clapham PJ, Alling A, O'Brien SJ, Palumbi SR (1993) Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. Proc natn Acad Sci USA 90: 8239–8243

Baker CS, Slade RW, Bannister JL, Abernethy RB, Weinrich MT, Lien J, Urbán J, Corkeron P, Clambokidis J, Vasquez O, Palumbi SR (1994) Hierarchical structure of mitochondrial DNA gene flow among humpback whales *Megaptera novaeangliae*, world-wide. Molec Ecol 3: 313–327

Birky CW, Fuerst P, Maruyama T (1989) Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics 121: 613–627

Birky CW, Maruyama T, Fuerst P (1983) An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics 103: 513–527

Bowen BW, Meylan AB, Ross JP, Limpus CJ, Balazs GH, Avise JC (1992) Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46: 865–881

Brown GG, Gadaleta G, Pepe G, Saccone C, Sbisa E (1986) Structural conservation and variation in the D-loop-containing region of vertebrate mitochondrial DNA. J molec Biol 192: 503–511

Buroker NE (1983) Population genetics of the American oyster *Crassostrea virginica* along the Atlantic coast and the Gulf of Mexico. Mar Biol 75: 99–112

Cann RL, Wilson AC (1983) Length mutations in human mitochondrial DNA. Genetics 104: 699–711

Christensen I, Haug T, Wiig Ø (1990) Morphometric comparison of minke whales *Balaenoptera acutorostrata* from different areas of the North Atlantic. Mar Mammal Sci 6: 327–338

Danielsdottir AK, Duke EJ, Arnason A (1992) Genetic variation at enzyme loci in North Atlantic minke whales, *Balaenoptera acutorostrata*. Biochem Genet 30: 189–202

Dillon MC, Wright JM (1993) Nucleotide sequence of the D-loop region of the sperm whale (*Physeter macrocephalus*) mitochondrial genome. Molec Biol Evolut 10: 296–305

Edgington ES (1987) Randomization tests. 2nd edn. Marcel Decker, New York

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791

Felsenstein J (1993) PHYLIP: phylogeny inference package. Version 3.5c. Department of Genetics, University of Washington, Seattle

Gatesy J, Yelon D, DeSalle R, Vrba ES (1992) Phylogeny of the Bovidae (Artiodactyla, Mammalia), based on mitochondrial ribosomal DNA sequences. Molec Biol Evolut 9: 433–446

Goto M, Pastene LA (1995) Population differentiation in the western North Pacific minke whales revealed by RFLP analysis of mitochondrial D-loop DNA. Paper SC/47/NP4 presented to the IWC Scientific Committee, June 1995

Hoelzel AR, Dover GA (1991) Genetic differentiation between sympatric killer whale populations. Heredity, Lond 66: 191–195

Hoelzel AR, Hancock JM, Dover GA (1991) Evolution of the cetacean mitochondrial D-loop region. Molec Biol Evolut 8: 475–493

Hori H, Bessho Y, Kawabata R, Watanabe I, Koga A, Pastene L (1994) World-wide population structure of minke whales deduced from mitochondrial DNA control region sequences. Paper SC/46/SH14 presented to the IWC Scientific Meeting, May 1994

Horwood J (1990) Biology and exploitation of the minke whale. CRC Press, Boca Raton, Florida

Hultman T, Ståhl S, Hornes E, Uhlen M (1989) Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. Nucleic Acids Res 17: 4937–4946

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian protein metabolism. Academic Press, New York, pp 21–123

Karl SA, Avise JC (1992) Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science, Wash 256: 100–102

Kocher TD, Thomas WK, Meyer A, Edwards SC, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc natn Acad Sci USA 86: 6196–6200

Kraus F, Miyamoto MM (1991) Rapid cladogenesis among the pecoran ruminants: evidence from mitochondrial DNA sequences. Syst Zool 40: 117–130

Lynch M, Crease TJ (1990) The analysis of population survey data on DNA sequence variation. Molec Biol Evolut 7: 377–394

Manly BFJ (1991) Randomization and Monte Carlo methods in biology. 2nd edn. Chapman and Hall, London

McKnight ML, Dodd CKJ, Spolsky CM (1991) Protein and mitochondrial DNA variation in the salamander *Phaeognathus hubrichti*. Herpetologica 47: 440–447

Meyer A, Kocher TD, Basasibwaki P, Wilson AC (1990) Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature, Lond 347: 550–553

Milinkovitch MC, Meyer A, Powell JR (1994) Phylogeny of all major groups of cetaceans based on DNA sequences from three mitochondrial genes. Molec Biol Evolut 11: 939–948

Moritz C (1994) Application of mitochondrial DNA analysis in conservation: a critical review. Molec Ecol 3: 401–411

Nei M (1982) Evolution of human races at the gene level. In: Bonne-Tamir B, Cohen P, Goodman RN (eds) Human genetics, Part A: the unfolding genome. Alan R. Liss, New York, pp 167–181

Omura H (1975) Osteological study of minke whale from the Antarctic. Scient Rep Whales Res Inst, Tokyo 27: 1–36

Palsbøll PJ (1989) Restriction fragment pattern analysis of mitochondrial DNA in minke whales, *Balaenoptera acutorostrata*, from the Davis Strait and the Northeast Atlantic. Chapter from Master's thesis, University of Copenhagen, Copenhagen

Palsbøll PJ, Clapman PJ, Mattila DK, Larsen F, Sears R, Siegismund HR, Sigurjónsson J, Vasquez O, Arctander P (1995) Distribution of mtDNA haplotypes in North Atlantic humpback whales: the influence of behaviour on population structure. Mar Ecol Prog Ser 116: 1–10

Palumbi SR, Baker CS (1994) Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. Molec Biol Evolut 11: 426–435

Palumbi SR, Martin AP, Kessing B, McMillan WO (1991) Detecting population structure using mitochondrial DNA. In: Hoelzel AR (ed) Genetic ecology of whales and dolphins. Report IWC, Special Issue 13, International Whaling Commission, Cambridge, pp 203–215

Palumbi SR, Wilson AC (1990) Mitochondrial DNA diversity in the sea urchins *Strongylocentrotus purpuratus* and *S. droebachiensis*. Evolution 44: 403–415

Pastene LA, Goto M, Itoh S (1995) Spatial and temporal patterns of mitochondrial DNA variation in minke whale from Antarctic areas IV and V. Paper SC/47/SH6 presented to the IWC Scientific Committee, June 1995

Rao VB (1994) Direct sequencing of polymerase chain reaction-amplified DNA. Analyt Biochem 216: 1–14

Reeb CA, Avise JC (1990) A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. Genetics 124: 397–406

Rosel PE, Dizon AE, Heyning JE (1994) Genetic analysis of sympatric morphotypes of common dolphins (genus *Delphinus*). Mar Biol 119: 159–167

Saccone C, Pesole G, Sbisa E (1991) The main regulatory region of mammalian mitochondrial DNA: structure–function model and evolutionary pattern. J molec Evolut 33: 83–91

Schaeff C, Kraus S, Brown M, Perkins J, Payne R, Gaskin D, Boag P, White B (1991) Preliminary analysis of mitochondrial DNA variation within and between right whale species *Eubalaena glacialis* and *Eubalaena australis*. In: Hoelzel AR (ed) Genetic ecology of whales and dolphins. Report IWC, Special Issue 13, International Whaling Commission, Cambridge, pp 217–223

Skåre M (1994) Whaling: a sustainable use of natural resources or a violation of animal rights? Environment, St. Louis, Mo 36 (7): 12–36

Taberlet P, Bouvet J (1994) Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear *Ursus arctos* in Europe. Proc R Soc (Ser B) 255: 195–200

van Piljen IA, Amos B, Burke T (1995) Pattern of genetic variability at individual minisatellite loci in minke whale *Balaenoptera acutorostrata* populations from three different oceans. Molec Biol Evolut 12: 459–472

Wada S (1991) Genetic heterogeneity in the Okhotsk Sea–West Pacific stock of minke whales. Paper SC/43/Mi32 presented to the IWC Scientific Committee, June 1991

Wada S, Kobayashi T, Numachi KI (1991) Genetic variability and differentiation of mitochondrial DNA in minke whales. In: Hoelzel AR (ed) Genetic ecology of whales and dolphins. Report IWC, Special Issue 13, International Whaling Commission, Cambridge, pp 203–251

Wada S, Numachi KI (1991) Allozyme analysis of genetic differentiation among the populations and species of the *Balaenoptera*. In: Hoelzel AR (ed) Genetic ecology of whales and dolphins. Report IWC, Special Issue 13, International Whaling Commission, Cambridge, pp 125–154

Wayne RK, Lehman N, Girman D, Gogan PJP, Gilbert DA, Hansen K, Peterson RO, Seal US, Eisenhawer A, Mech LD, Krumenaker RJ (1991) Conservation genetics of the endangered isle royal gray wolf. Conserv Biol 5: 41–51

Wolstenholme DR (1992) Animal mitochondrial DNA: structure and evolution. In: Wolstenholme DR, Jeon KW (eds) Mitochondrial genomes. Academic Press, San Diego, pp 173–216