Phylogenetic analysis of fin whale mtDNA control region sequences world-wide

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ABSTRACT

We examined the genetic relationship of fin whales among different ocean basins based on the variation in the nucleotide sequence of the mitochondrial control region using samples obtained from western and eastern North Pacific, North Atlantic and Antarctic oceans. Samples were collected from Antarctic Ocean (n=9) as biopsy samples and from western North Pacific Ocean (n=5) as biopsy as well as stranded and by-caught whales. In addition to these samples, 48 published haplotypes from North Atlantic Ocean and the Mediterranean Sea and seven haplotypes from eastern North Pacific and the sea of Cortez also used for a phylogenetic analysis based on neighbor-joining and parsimony. A common 288bp fragment of the mtDNA control region was used. A total of 66 haplotypes were determined in the total samples. All samples from Antarctic and North Pacific grouped in the same clade. This clade also included two haplotypes detected in the North Atlantic samples. No clade specific to a single ocean basin was found, indicating little time since their divergence to accumulate unique haplotypes. Our study also indicates that assigning mtDNA haplotypes examined from fin whales of unknown origin is difficult.

INTRODUCTION

Up to date, the phylogenetic analyses among different ocean basins were examined for some baleen whales, such as humpback whale (Baker *et al.* 1993, Baker *et al.* 1994), minke whale (Pastene *et al.* 2001) and right whale (Rosenbaum *et al.* 2000). As regard to fin whales, however, there were no phylogenetic analysis among ocean basin other than allozyme analysis by Wada and Numachi (1991).

As early as 1960, Fujino (1960) had used blood typing as well as marking and morphological data to identify three 'sub-populations' of fin whales in the North Pacific: the East China Sea (a small local stock) and two stocks on the eastern and western side of the Aleutians.

Wada and Numachi (1991) conducted allozyme analysis using North Pacific, Spanish coastal and Antarctic fin whales. They showed significant allele frequency differences between Hemispheres. However they could not detect evidence of more than one stock in the Antarctic and North Pacific fin whales.

Bérubé *et al.* (1998) conducted fin whale population genetic study based on the analysis of the mtDNA control region as well as six microsatellite loci of 407 fin whale skin samples originating from the North Atlantic Ocean, the Mediterranean Sea, the Sea of Cortez and off the coast of California (eastern North Pacific Ocean). The study proposed segregation on the feeding as well as breeding grounds with limited amount of gene flow between adjacent populations. Furthermore, it is suggested that fin whales in the North Atlantic are separate from those inhabiting the Mediterranean Sea. In addition, they showed that the degree of genetic diversity among fin whales in the Sea of Cortez at nuclear and mitochondrial loci was exceptionally low. Following this study, Bérubé *et al.* (2002) analyzed geographic distribution of genetic variation at a mtDNA control region and 16 nuclear microsatellite loci in samples collected from fin whales in the eastern North Pacific as well as Sea of Cortez. They found that fin whales observed in the Sea of Cortez were highly isolated and thus an evolutionary unique population.

In this study, we presents the results of a genetic relationship of fin whales among different ocean basin based upon the variation in the nucleotide sequence of the mitochondrial control region using the samples from western and eastern North Pacific, North Atlantic and Antarctic oceans.

MATERIALS AND METHOD

Samples

Samples were collected from summer feeding areas in the Antarctic Area V (n=9) and in the western North Pacific (n=5) (Table 1 and Fig.1). All samples from Antarctic and one sample from western North Pacific were obtained as skin biopsies during JARPA (Japanese Whale Research Program under Special Permit in the Antarctic) and JARPN (Japanese Whale Research Program under Special Permit in the North Pacific), respectively. Another four samples from western North Pacific were collected from stranded or by-caught animals in the coast of Sea of Japan (n=2), Tsugaru Strait (n=1) and Inland Sea of Japan (n=1). All samples were stored at either –20°C and –80°C pending analysis. In addition to these samples, 55 fin whale haplotypes of North Atlantic and North Pacific fin whales (Bérubé *et al.* 1998, Bérubé *et al.,* 2002) were examined in this study for comparison.

Molecular Analyses

Sequencing of the mtDNA control region

Using established protocols (Sambrook *et al.*, 1989), total-cell DNA was extracted from skin tissue samples. The first half of control region of the mitochondrial genome was amplified by using the polymerase chain reaction (PCR). In order to amplify the approximately 500 bp minke whale mtDNA including control region, primers light-strand MT4 (*5'*-CCTCCCTAAgACTCAAggA-Ag-*3'*) and heavy-strand Dlp 5R (*5'*-CCATCgAgATgTCTTATTTAAggggAAC-*3'*) were used. PCR products were purified by MicroSpin S-400HR columns (Pharmacia Biotech). Cycle sequencing was performed with the same primers, using AmpliTaq FS Sequencing Kit (Perkin-Elmer, Inc). The cycle sequencing products were purified by AutoSeq G-50 spin Columns (Pharmacia Biotech). The labeled sequencing fragments were resolved by electrophoresis through a 5% denaturing polyacrylamide matrix on an ABI 377™ Automated DNA Sequencer (Applied Biosystems, Inc), following the protocols of the manufacture. For each sample both strands were sequenced.

Data analysis

Level of polymorphism

The level of diversity in fin whale using mtDNA control region sequences was estimated at nucleotide level. The nucleotide diversity (Nei, 1987: equation 10.5) and its standard error for population sampling and stochastic processes were calculated from the pairwise differences between the mtDNA sequences using the Kimura's 2- parameter adjustment (Kimura, 1980).

MtDNA Phylogeny

Sequences were aligned initially using Sequence Navigator (Applied Biosystems, Inc). The aligned sequences were then corrected by eye for minor inconsistencies. Phylogenetic reconstruction of sequences was made using neighbor-joining method implemented in the computer package PHYLIP version 3.5c (Felsenstein, 1995) and parsimony methods generated using PAUP* v. 4.0b10 (Swofford, 2001). Genetic distance among haplotypes were estimated using the program DNADIST of the Phylip computer package based on Kimura's 2- parameter model (Kimura, 1980) and an observed transition:transversion ratio of 20:1. The phylogenies were rooted using the homologous sequence from Antarctic minke whale. The bootstrap values were obtained by generating 1000 random samples, for which distance matrices were also computed (Kimura 2-parameter model). Finally a majority-rule consensus phylogeny was calculated from the resultant 1000 phylogenies.

RESULTS AND DISCUSSION

Although the first 480 bp at the 5' end of the mtDNA control region was sequenced in the samples from eastern North Pacific (n=5) and Antarctic (n=9) Oceans, only the common 288 bp between these samples and those used in previous studies (Bérubé *et al.*, 1998, Bérubé *et al.,* 2002) were analyzed. Thirty-seven polymorphic sites were detected, 36 of which were transitions and one transversion. No insertion/deletion events were observed. The 37 polymorphic sites defined 66 unique haplotypes. In the western North Pacific samples, NPM101 had haplotype SCBp49, originating from the Sea of Cortez and NPB97F01 corresponded to haplotype NPBp54, originating from the eastern North Pacific (Bérubé, *et al.* 1998; Bérubé *et al.* 2002). Antarctic ANT00VF06 and ANT00VF08 had same haplotype.

Estimated nucleotide diversities were 0.0134 (standard error 0.0012) and 0.0136 (standard error 0.0030) for Antarctic and among western North Pacific, respectively. Although sample sizes in these

areas were small, these values were higher than the estimate obtained from two eastern North Pacific samples (Coastal California; 0.0058 (standard error 0.0018), Sea of Cortez; 0.00059 (standard error 0.0002)) (Bérubé *et al.* 2002), but lower than that of over all North Atlantic samples 0.113 (standard error 0.0006) (Bérubé *et al.* 1998).

The two genealogies estimated using the parsimony and the neighbour-joining method yielded similar topologies. For this reason, only the majority-rule consensus tree of neighbor-joining method with bootstrap values above 50 % was presented except for one node with bootstrap values near-to 50% (48%) as shown in Fig. 2.

The tree showed that most of the haplotypes found in North Atlantic grouped together into several clades. Although not supported by high bootstrap value (48%), a clade grouped haplotypes from the North Pacific and Antarctic fin whales together. However, these samples were not further clustered into different clades, suggesting little time for fin whales inhabiting different ocean basin since their divergence to accumulate own unique haplotypes.

It is also important to mention that this cluster involved two haplotypes (NABp07 and NABp39) detected in North Atlantic. Our result, therefore, point out that assignment of mtDNA haplotypes examined from fin whales of unknown origin is difficult with only using their phylogenetic relationship especially based on sequence variation at mtDNA control region.

Other interesting aspect in this analysis is that individual NPM101 from eastern North Pacific (Sea of Japan) had haplotype SCBp49 that was found in and was accounted for 90% of individuals sampled from the Sea of Cortez. Furthermore, NPB97F01 biopsied from western North Pacific had haplotype NPBp54 that was private haplotype of Coastal California in Bérubé *et al.* (2002). Bérubé *et al.* (2002) found that the Sea of Cortez constituted a highly isolated, evolutionary unique population with very low degree of genetic diversity. Even though the low degree of genetic variation observed among fin whales in the Sea of Cortez could not preclude the possibility that this population could constitute part of a much larger eastern North Pacific fin whale population with equally low levels of genetic variation due to historical event, such as past founder effects or bottlenecks, the study showed clearly that the fin whales observed in the Sea of Cortez constitute a genetically isolated population with a small effective population size evident by low levels of genetic variation at nuclear as well as mitochondrial loci Therefore, the sharing of haplotype between the sample NPM101 (eastern North Pacific) and the haplotype SCBp49 (Sea of Cortez), suggests that "the recurrent gene flow" observed here probably reflect historical gene flow prior to the isolation of the Sea of Cortez from the North Pacific Ocean. Although we cannot exclude the possibility of ongoing gene flow or occasional movement of whales within North Pacific, these observations again suggest little time since their divergence. Sharing of haplotypes among the different ocean basin might, therefore, simply reflect residue of ancestral polymorphism.

Rosenbaum *et al.* (2000) showed that the combined phylogeographic, phylogenetic and population aggregation analyses all clearly demonstrate the differentiation of maternal lineages among right whales world-wide. Pastene *et al.* (2001) examined phylogenetic relationship among four morphological forms of minke whale from world-wide: North Atlantic, North Pacific and Southern Hemisphere ordinary and dwarf forms. They found that individuals from the four morphological types defined four corresponding clusters. Consequently, molecular characters showed that diagnostic characters exist for animals of each ocean in these two species.

The demographic or genetic isolation of oceanic populations of humpback whales was described from RFLP and control region sequences of mtDNA (Baker *et al.* 1993, Baker *et al.* 1994). Both methods indicate that humpback whale maternal lineages were highly subdivided among the three major oceanic populations despite the species' nearly unlimited dispersal potential. Among fin whales, as well as humpback whales, there are no diagnostic molecular characters in the mtDNA control region that identify whales by a region or geographical area.

Even if mtDNA dose not permit the assignment of individuals of unknown origin to different Ocean basins, the addition of nuclear DNA, such as microsatellite of SNPS (single nucleotide polymorphisms), might help to clearly distinguish between populations as it found in the Bérubé *et al.* (2002).

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Table 1 Summary of samples used in this study.

Code	Ocean	Sampling Period	Remark
NABp01-48	North Atlantic and Mediterranean Sea	1982-1994	Bérubé *et al.* (1998)
NABp49-55	Eastern North Pacific and Sea of Cortez	1993-1996	Bérubé *et al.* (1998, 2002)
NPM101	Western North Pacific (Sea of Japan)	1996	Present study
NPM102	Western North Pacific (Sea of Japan)	1996	Present study
NPM124	Western North Pacific (Tsugaru Strait)	1997	Present study
NPM228	Western North Pacific (Inland Sea)	2001	Present study
NPB97F01	Western North Pacific	1997	Present study
ANT98VF01	Antarctic (Area V)	1999	Present study
ANT98VF02	Antarctic (Area V)	1999	Present study
ANT98VF03	Antarctic (Area V)	1999	Present study
ANT00VF04	Antarctic (Area V)	2001	Present study
ANT00VF05	Antarctic (Area V)	2001	Present study
ANT00VF06	Antarctic (Area V)	2001	Present study
ANT00VF07	Antarctic (Area V)	2001	Present study
ANT00VF08	Antarctic (Area V)	2001	Present study
ANT00VF09	Antarctic (Area V)	2001	Present study

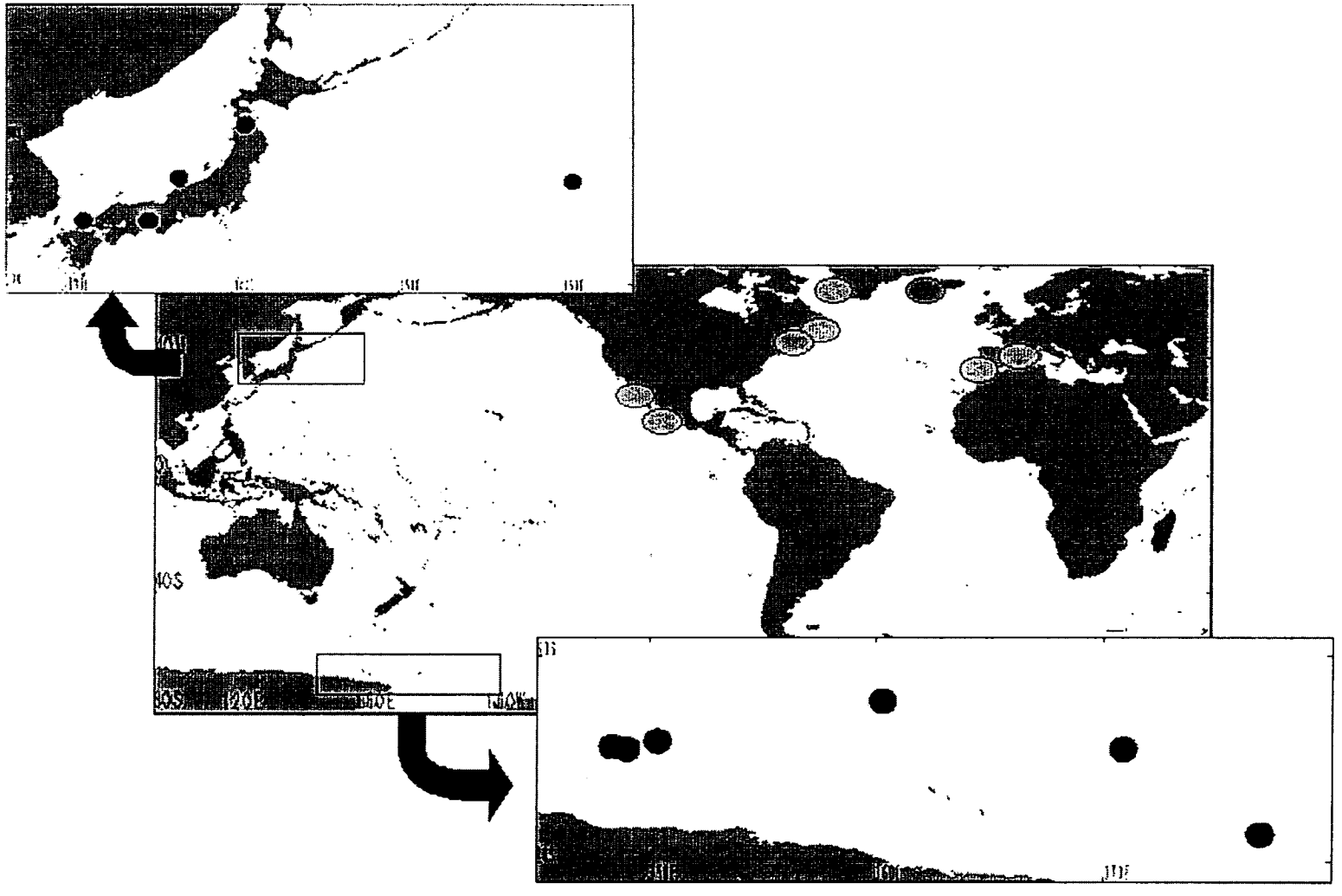

Fig. 1 Geographical sampling localities in the western North Pacific and Antarctic. Sampling areas in the eastern North Pacific and North Atlantic were cited from Bérubé *et al.*,(1998, 2002)

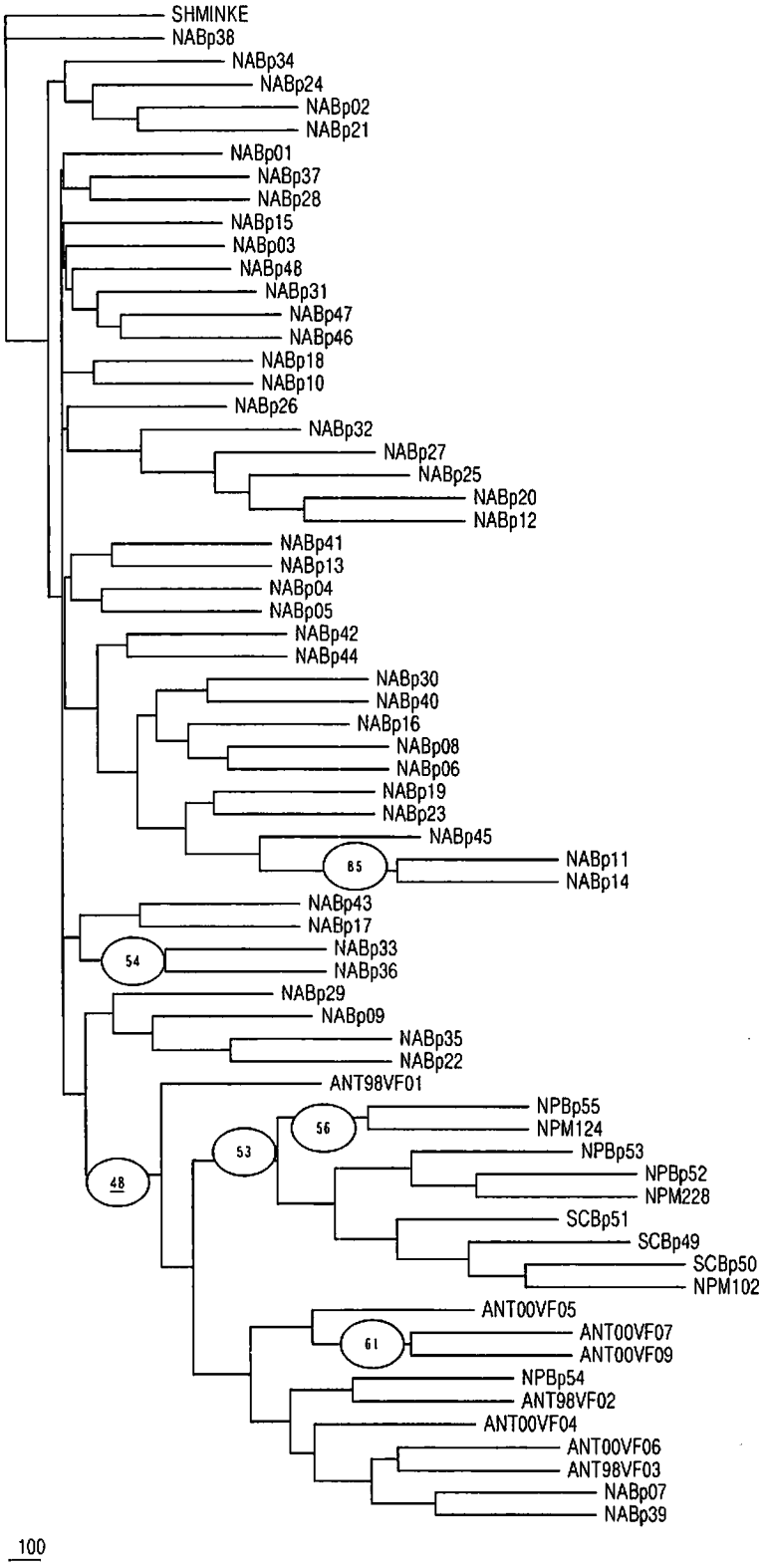

Fig. 2. Majority rule consensus tree estimated from mtDNA haplotypes using a neighbor-joining method. Only the bootstrap values above 50 % are shown except for one node with bootstrap values near-to 50% (48%).