Mitochondrial DNA variation in the Japanese harbour porpoise (*Phocoena phocoena*)

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Introduction

The harbour porpoise (*Phocoena phocoena*) is a small cetacean distributed in temperate and subarctic waters of the Northern Hemisphere. They have three major distribution areas, the North Atlantic, North Pacific and Black Sea to Sea of Azof (Fig.1, Gaskin, 1984).

They are top predators in coastal ecosystems, but today, are exposed to environmental pollutants and fisheries pressures (Jefferson and Curry, 1994; Beineke *et al.*, 2005; Jepson *et al.*, 2005). Especially, population reduction due to incidental catch has been a growing serious problem in a part of the North Atlantic (Woodley and Read, 1991; Trippel *et al*., 1996). It is important to understand their intraspecific genetic structure for appropriate management of harbour porpoises in such an environment.

In the North Pacific, two major local populations are known in the north and south coasts of North America, in the area designating the Northeast Pacific (Rosel *et al*., 1995). Moreover, some subpopulations in these two regions have been reported (Chivers *et al*., 2002). However, it is difficult to understand the genetic structure across the North Pacific because no population genetics study has been conducted in the Northwest Pacific, including Japan.

The Japanese harbour porpoise is distributed around Hokkaido to the northern mainland of Honshu in winter and east of Hokkaido to the Sea of Okhotsk in summer (Gaskin *et al*., 1993). Given the breeding season of the Japanese harbour porpoise, the Sea of Okhotsk is likely to be important as their breeding area. Thus, the present study is aiming to estimate the mitochondrial DNA variation of harbour porpoises in Japanese waters as a first step to understanding their genetic structure in the Northwest Pacific.

Materials and Methods

Samples and DNA extraction

A total of 56 tissue samples were obtained from stranded, incidentally caught or captive porpoises in Japan (Fig. 2). Tissues of muscle or skin were preserved in 95% ethanol or stored at –30°C. Total genomic DNA was extracted using Gentra Puregene kits (QIAGEN) following the manufacturer's protocol.

In addition, all nucleotide sequences in the Northeast Pacific of 40 individuals from Alaska and Strait of Georgia (NEP5), 52 individuals from San Juan Island and Vancouver Island (NEP4), 62 individuals from Washington and Columbia River (NEP3), 17 individuals from Oregon (NEP2), and 52 individuals from San Francisco and Monterey Bay (NEP1) (accession numbers AF461818 – AF461891, Chivers *et al.*, 2002) were collected from GenBank.

Mitochondrial control-region sequences

The 462 base pairs (bps) of the hypervariable portion from the 5' end of the mitochondrial (mt) DNA control region were amplified using polymerase chain reaction with primer L15824 (5'-CCTCACTCCTCCCTAAGACT-3') and H16265 (5'-GCCCGGTGCGAGAAGAGG-3') (Rosel *et al*., 1999). A reaction mixture at 20 μL contained 2 μL of 10x buffer, 0.25 μL of each primer, 0.15 mM dNTP and 0.02 U/μL *Taq* DNA polymerase. Amplification was carried out on an ASTEC PC320, 708 or 816 thermal cycler with a thermal profile consisting of an initial denaturation at 95°C for 30 s, followed by 30 cycles of 50 s at 94°C, 50 s at 55°C and 50 s at 72°C, and a final extension at 72°C for 5 min. The amplified products were purified using a DNA purification kit, and cycle-sequenced using an ABI BigDye® Terminator v3.1 cycle sequencing kit,

following the manufacturer's protocol. All samples were sequenced in both DNA strands with the primers used in the amplification, and sequence alignment was corrected by eye.

Analysis of mitochondrial DNA variation

Nucleotide and haplotype diversities (Nei, 1987) were estimated for all populations. An analysis of molecular variance (AMOVA) was conducted to measure the hierarchical genetic differentiation among populations using the program ARLEQUIN ver.3.1 (Excoffier and Schneider, 2005). The AMOVA calculates ϕ_{ST}, an index of population subdivision. The significance of ϕ_{ST} was tested by multiple permutations of the original data sets. A phylogenetic relationship among mtDNA haplotypes was performed with the neighbor-joining (NJ) method on a matrix of the pairwise distance, based on Kimura's two-parameter model (Saitou and Nei, 1987), using the finless porpoise (*Neophocoena phocaenoides*) as out group, with the program MEGA ver.4 (Tamura *et al.*, 2007). The reliability of internal tree branches was assessed by 1,000 bootstrap resamplings. A minimum spanning network of mtDNA haplotypes was constructed using the program TCS ver.1.21 (Clement *et al.*, 2000). Isolation by distance, known as the population genetic pattern, was estimated by regression analysis between ϕ_{ST} and the geographic distance among all pairwise populations, using Isolation by Distance Web Service (Jensen *et al*., 2005).

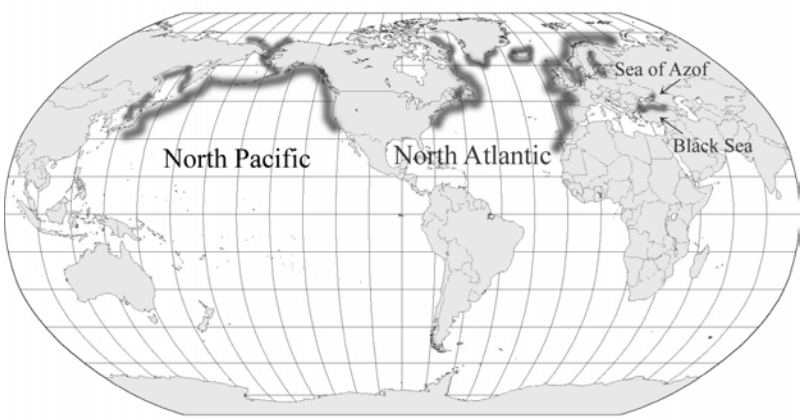

Fig. 1 Three distribution areas of the harbour porpoise.

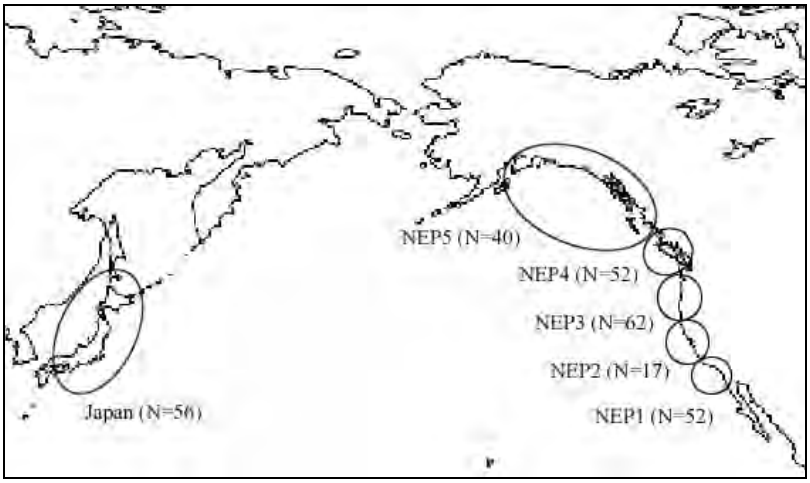

Fig. 2 Geographic locations and number of harbour porpoise specimens or sequences used in this study.

Results

Sequence analysis of 358 bps in the 5' 462 bps sequenced portion of the mtDNA control region revealed 23 variable sites, which defined 19 haplotypes among Japanese porpoises (Table 1).

Haplotype and nucleotide diversities were 0.78 and 0.006, respectively, in Japan. The haplotype diversity ranged from 0.64 to 0.91 and nucleotide diversity ranged from 0.006 to 0.016. Both diversities were lower in Japan and NEP5 than the other populations, and became higher toward south of the Pacific Rim. The reduced value in NEP2 might be caused by small sample size.

In the NJ tree for a total of 67 haplotypes, two geographic clusters, groups I and II, were confirmed, but not divided completely (Fig. 3). Although some haplotypes were endemic to the Japanese population, many other haplotypes were distributed across a broad geographic range and the reliability values of the tree were low.

A minimum spanning network resolved two main haplotype groups (groups I and II, Fig. 4), and these two groups were compatible with those of the NJ tree (Fig. 3). The Northeast Pacific populations had both haplotype groups, but the Japanese population had only group I. The most common haplotype, haplotype 49, was found in all sampling locations and was focal in a star-like group I genealogy. The second most common haplotype, haplotype3, was focal in the group II.

Table 1 Genetic diversity indices in 6 locations.

	Japan	NEP5	NEP4	NEP3	NEP2	NEP1
No. of samples	56	40	52	62	17	52
No. of polym. sites	23	25	35	33	17	30
No. of haplotypes	19	14	19	23	8	18
Haplotype diversity	0.78	0.64	0.88	0.91	0.73	0.90
Nucleotide diversity	0.006	0.007	0.015	0.015	0.011	0.016

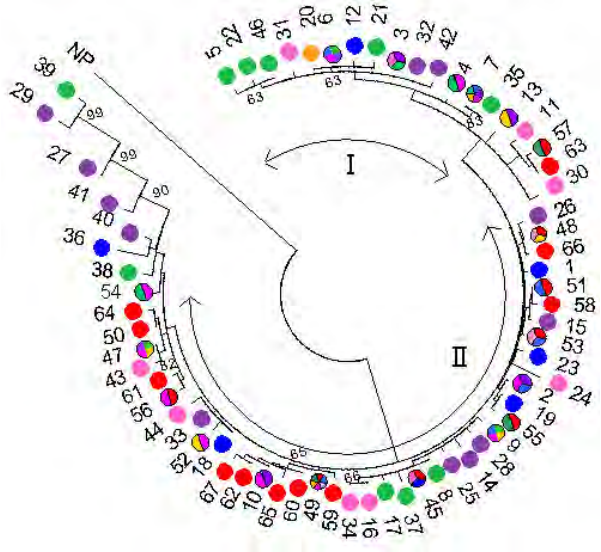

Fig. 3 Neighbor-joining tree for 76 haplotypes of harbour porpoises in the North Pacific. The nodal numbers indicate bootstrap support over 60% in 1000 replications. The circle colors show those haplotypes found in the following regions: red (Japan); blue (NEP5); violet (NEP4); pink (NEP3); orange (NEP2); green (NEP1).

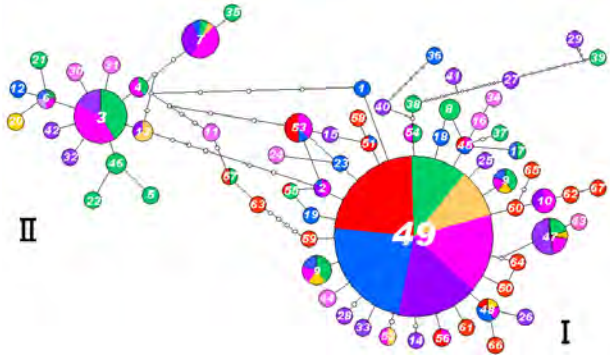

Fig. 4 The parsimony network for a total of 67 haplotypes of the harbour porpoise in the North Pacific. The numbers in the circles indicate haplotype ID. Circle size reflects the haplotype abundance and the color of the sampling locales referred to Figure 3. Small open circles are intermediate haplotypes.

The AMOVA showed a significant difference for ϕ_{ST} between Japan and the Northeast Pacific, except for NEP5, which was significantly differentiated from the other populations, except for Japan and NEP2 (Table 2). An insignificant value between NEP5 and NEP2 may be due to the small sample size in NEP2. Overall, differentiation increased southward in the Pacific Rim.

There was significant correlation between genetic distance for ϕ_{ST} and geographic distance among all pairwise populations (Fig. 5). This relationship suggests a restricted gene flow by distance in the North Pacific.

Discussion

Although two main geographic clusters were confirmed on the NJ tree of haplotypes in the harbour porpoise, its branching reliability was not high with weak to moderate bootstrap support. In addition, most of the observed haplotypes were distributed across a broad geographic range, although some were endemic to Japan. These results suggest the occurrence of a certain level of gene flow over the distribution range in the North Pacific.

A minimum spanning network also resolved two haplotype groups. A star-like genealogy of both haplotype groups suggests recent population expansion of the harbour porpoise in the North Pacific, as a whole. Most populations in the Northeast Pacific had these two groups. Although the Japanese population had only group I haplotypes, AMOVA did not show significant differentiation between Japan and NEP5, Alaska to Strait of Georgia. Moreover, there was a significantly positive relationship between genetic and geographic distances, suggesting a restricted gene flow with isolation by distance.

Both the genetic differentiation and haplotype diversity data obtained may indicate a contiguous expansion of their range from south to north along the North Pacific Rim, and the occurrence of the Japanese population in the Northeast Pacific populations after bottleneck. This also is supported by lower genetic diversities north of the Pacific Rim. Rosel *et al*. (1995) also reported lower genetic diversity of the harbour porpoise in the northern location of the Northeast Pacific, and they inferred that this phenomenon reflected historical colonization by a small group having a part of their lineage in an ancestral population.

No difference between the Japan and Alaska to the Strait of Georgia population despite the very long distance is puzzling. The Sea of Okhotsk may become a key area to resolving this problem. However, the role of the Sea of Okhotsk on gene flow of the harbour porpoise in the Northwest Pacific remains unknown because there are no population genetic data in the Bering Sea, around the Aleutian Islands and coastal Russia. In the future, studies using the data from these areas will help in appropriate management of the harbour porpoise in the Northwest Pacific.

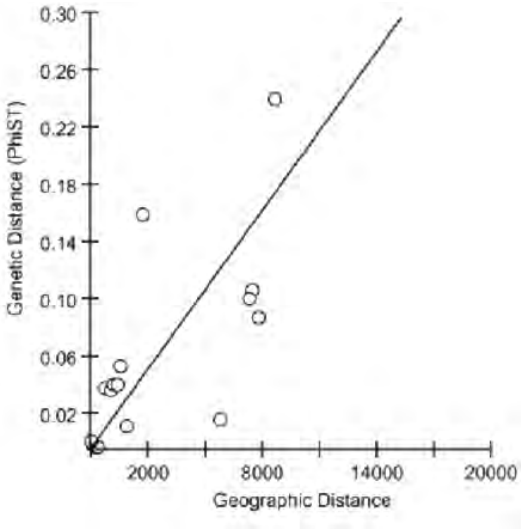

Fig. 5 Correlation between ϕ_{ST} and geographic distance among populations in the North Pacific.

Table 2 Pairwise population ϕ_{ST} with statistical significance ($p < 0.05$, below diagonal).

	Japan	NEP5	NEP4	NEP3	NEP2	NEP1
Japan		0.019	0.104	0.153	0.083	0.247
NEP5	–		0.048	0.097	0.016	0.175
NEP4	+	+		0.005	–0.0022	0.0147
NEP3	+	+	–		–0.005	0.013
NEP2	+	–	–	–		0.047
NEP1	+	+	+	–	–	

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