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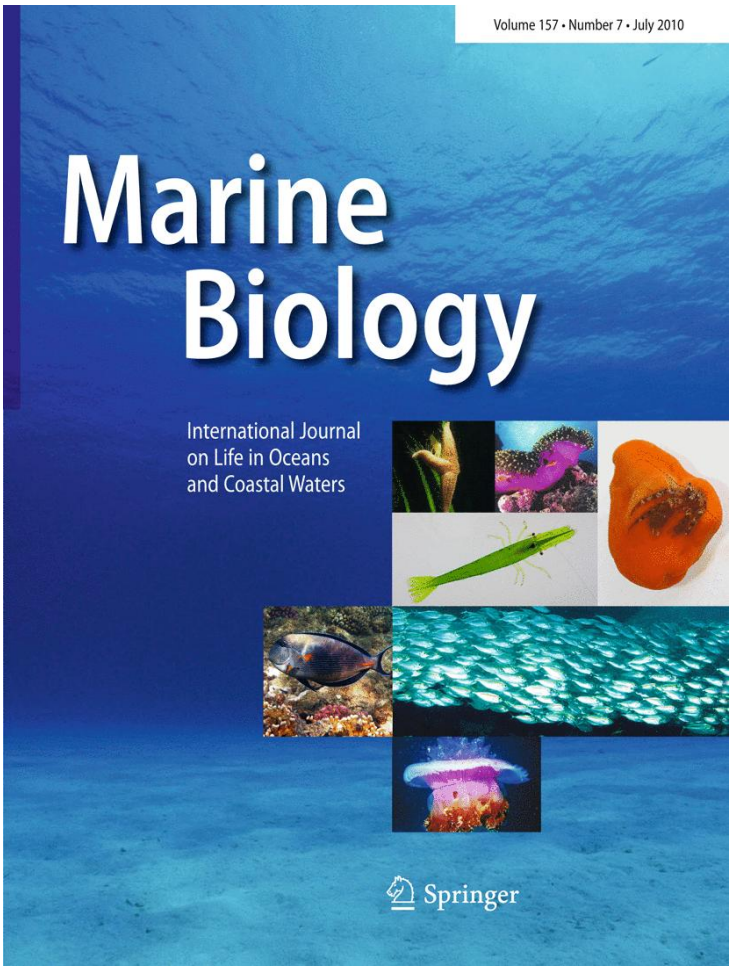

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ORIGINAL PAPER

Mitochondrial DNA phylogeography of the harbour porpoise *Phocoena phocoena* in the North Pacific

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Abstract Genetic structure and phylogeography of the harbour porpoise *Phocoena phocoena* in the North Pacific were examined using 358 bps sequences from the 5′ end of the mitochondrial DNA control region including those reported previously and newly obtained from the west Pacific. AMOVA and pairwise population φ_{ST} estimates clearly revealed genetic differentiation between an east/south and a north/northwest group with the break along the Pacific Rim at British Columbia. In addition, nested clade phylogeographical analysis, neutrality tests, mismatch distribution analysis, genetic diversities and Mantel test, suggested that the observed genetic structure might have been influenced by contiguous range expansion with restricted gene flow in the direction from south to north along the North American coasts and east to west along the Pacific Rim in the middle to late Pleistocene.

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Introduction

Many marine species show surprizingly fine genetic differentiation despite their high dispersal ability and the lack of obvious geographical barriers in the marine environment (Palumbi 1994). Therefore, studies of genetic differentiation in highly mobile organisms with vast distribution ranges like cetaceans will contribute to understanding of the genetic structuring and historical population process observed in marine animals. Although global climate conditions in the Quaternary are thought to have impacted on the genetic structure and composition for many taxa (Hewitt 2000), evolutionary process of marine mammals including cetaceans mostly remains obscure.

Among cetaceans, the family Phocoenidae, the true porpoise, includes six extant species, five of which inhabit primarily nearshore waters. The harbour porpoise *Phocoena phocoena* is such a coastal phocoenid, having a circumpolar distribution in temperate regions of the northern hemisphere, where no other coastal odontocete is distributed as widely. Morphological and genetic data suggest that globally, the harbour porpoise is comprised of at least three genetically isolated populations: the North Pacific, the North Atlantic and the Black Sea/Sea of Azov (Amano and Miyazaki 1992; Rosel et al. 1995). The genetic structure of harbour porpoises has been well studied using mainly mitochondrial (mt) DNA, particularly in the North Atlantic (e.g. Tolley and Rosel 2006; Rosel et al. 1999a, 1999b), because of serious reductions in the population size caused by incidental catch (e.g. Jefferson and Curry 1994). On the other hand, genetic studies have only been conducted in the eastern North Pacific (Rosel et al. 1995; Chivers et al. 2002), although the harbour porpoise ranges along the Pacific Rim from California to northern Japan (Gaskin 1984). The eastern North Pacific genetic studies suggested

the harbour porpoise population is subdivided into relatively small demographically isolated populations (Chivers et al. 2002). Morphological analysis also suggested differentiation of harbour porpoise populations between the eastern and western North Pacific (Amano and Miyazaki 1992). However, the phylogeographic concordance in the pattern of genetic differentiation across the North Pacific remains unknown.

The phylogeography of this species in the North Pacific should provide insight not only into understanding of the process of population differentiation for a coastal species with high dispersal capabilities, but also aid the current situation of genetic structure and diversity of the harbour porpoise in the North Pacific. This information may, in turn, aid conservation and management of the species in areas where it is exposed to high mortality rates due to various human activities.

The present study thus aimed at examining the phylogeography of the harbour porpoise from the North Pacific using variation of nucleotide sequences in the mtDNA control region, including those reported previously and newly obtained in the present study.

Materials and methods

Samples and DNA extraction

Tissue samples were obtained from a total of 59 stranded, incidentally caught or captive porpoises, which included 58 from Japan and one from British Columbia. Some of Japanese samples were stored in the Institute of Cetacean Research, Tokyo, Ehime University, Ehime, and the National Science Museum, Tokyo. Total genomic DNA was extracted from a piece of muscle or skin preserved in 95% ethanol at room temperature or stored frozen at −30°C and from freshly obtained blood, using Gentra Puregene kits (QIAGEN) following the manufacturer's protocol.

Nucleotide sequence analysis of the mtDNA control region

About 470 base pairs (bps) of the hypervariable region from the 5′ end of the mtDNA control region and flanking tRNA genes were amplified using the polymerase chain reaction (PCR) with primers L15824 (5′-CCTCACTCCT CCCTAAGACT-3′) and H16265 (5′-GCCCGGTGCGA GAAGAGG-3′) (Rosel et al. 1999b). Twenty microlitres of reaction mixture contained 2 µl of 10x buffer (10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$), 0.4 µM each of primers, 0.15 mM of dNTPs and 0.5 unit of *Taq* DNA polymerase. Amplification was carried out on ASTEC PC320, 708 or 816 thermal cycler with a thermal profile consisted of a pre-cycling 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, with a post-cycling extension for 5 min at 72°C. PCR products were purified using a DNA purification kits (Beckman Coulter), and cycle-sequenced using an ABI BigDye® Terminator v3.1 cycle sequencing kit (Life Technologies) following the manufacturer's protocol. All samples were sequenced for both strands with the primers used in amplification. The obtained sequences were edited and then aligned with the GENETYX ver.5.1.1 (Genetyx Corporation).

Analysis of genetic differentiation and historical demography

Genetic differentiation and historical demography of the harbour porpoise in the North Pacific were analyzed using our new sequence data from the western, i.e. Japan, and eastern North Pacific, i.e. British Columbia, and those obtained previously (Fig. 1; Rosel et al. 1995; Chivers et al. 2002). Rosel et al. (1995) examined the population structure of the harbour porpoises in California, Washington, British Columbia and Alaska using 81 mtDNA control region sequences, and Chivers et al. (2002) analyzed the population structure of the eastern North Pacific using 225 of 249 control region sequences which were different from those used by Rosel et al. (1995). In the present study, a novel stratum of British Columbia was created by including nine from 24 sequences excluded by Chivers et al. (2002), because of too few to define a stratum and/or too

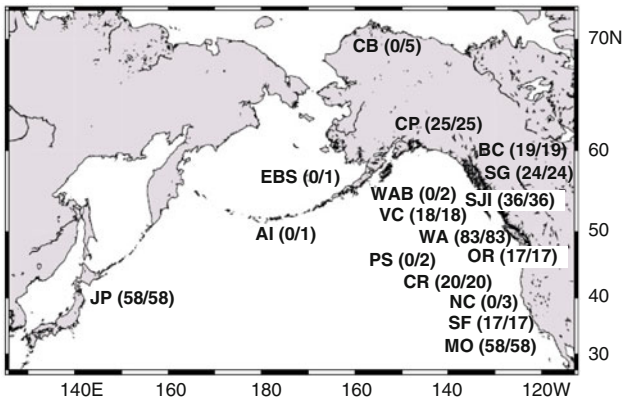

Fig. 1 Sampling locales of the harbour porpoise used in the present study. The sample sizes by strata for each analysis were given following stratum ID (genetic structuring and historical demography/NCPA). The abbreviations of stratum ID were shown as *MO* Monterey; *SF* San Francisco; *NC* North California; *OR* Oregon; *CR* Columbia River; *WA* Washington; *VC* Vancouver; *SJI* San Juan Islands; *PS* Puget Sound; *SG* Strait of Georgia; *BC* British Columbia; *CP* Copper River; *WAB* western Alaska Bay; *EBS* eastern Bering Sea; *CB* Cape of Barrow; *AI* Aleutian Islands; *JP* Japan

distant to allocate into strata, and nine of the 81 sequences used by Rosel et al. (1995). In addition, one sequence from the Lopez Island (Chivers et al. 2002) was included in a stratum of the San Juan Islands. Including the above-mentioned two new strata, Japan and British Columbia, 11 strata in accordance with a priori definition (e.g. a distributional hiatus in sampling or geographic barriers; Chivers et al. 2002) were assigned for the analysis of genetic differentiation. The remaining 14 sequences from Chivers et al. (2002) were used for historical demographic analyses to be hereinafter described. Thus, a total of 316 and 330 sequences were collected from the previous studies for the present genetic differentiation and historical demographic analyses, respectively (Rosel et al. 1995; Chivers et al. 2002; GenBank accession numbers AF461818–AF461891; see also supplementary material). No sequence data in the Bering Sea, coastal Aleutian Islands and Russia were available for the present analyses.

An analysis of molecular variance (AMOVA) was conducted to measure the hierarchical genetic differentiation among strata and the significance of pairwise conventional F_{ST} and φ_{ST} values, each an index of population subdivision, based on the Tamura-Nei model of evolution (Tamura and Nei 1993) with gamma correction ($\alpha = 0.30$; Tolley et al. 2001) using 10,000 random permutations of the original data sets. Statistical significance of pairwise φ_{ST} values were used to break the strata into two groups—a east/south group including all samples from Monterey to the British Columbia and a north/northwest group containing samples from the Strait of Georgia, Alaska and Japan. A pattern of isolation by distance was also assessed by testing the correlations between geographic distance and Nei's average number of differences between populations (D_A) (Nei and Li 1979) as a measure of genetic distance, using the Mantel test (Mantel 1967) with 10,000 permutations. The geographic distance of coastal line between neighbouring sampling sites were measured in the geographic information system software Arc GIS ver. 9.3 (ESRI). Nucleotide and haplotype diversities (Nei 1987) were estimated for each stratum and group detected by the significant φ_{ST} values described earlier. In order to infer past demographic changes, Tajima's D-statistic (Tajima 1989), Fu's F_S (Fu 1997) and R_2 statistics (Ramos-Onsins and Rozas 2002) were used to test for selective neutrality for each stratum and group. Statistical significance was determined based on 10,000 coalescent simulations. Mismatch distribution analysis (Rogers and Harpending 1992; Excoffier 2004) was also conducted for each group to reveal whether they experienced a historical population expansion. The observed distribution of pairwise nucleotide differences among individuals was compared to the expected distributions under the sudden expansion model for each group, and the goodness-of-fit tests (sum of squared deviations, SSD; Schneider and Excoffier 1999) of the observed to the simulated mismatch distributions were computed. The approximate dates of demographic changes for each group were calculated by the formula $T = \tau/2\mu$, where T is time in generations, τ is the date of the expansion simulated in the model of demographic expansion, and μ is the mutation rate for the whole sequence assayed (Rogers and Harpending 1992). A mutation rate of ca. $\mu = 3.3$–4.3% per site per million year was used, as a standard evolutionary rate for the sequenced portion of the mtDNA control region of the harbour porpoise as estimated previously (Tolley and Rosel 2006). The program ARLEQUIN ver. 3.1 (Excoffier et al. 2005) and DnaSP ver. 4.50.3 (Rozas et al. 2003) were used for all the above-mentioned analyses.

Nested clade phylogeographical analysis (NCPA)

NCPA was conducted by ANeCA v1.2 (Panchal 2007), which is a fully automated implementation of NCPA, including TCS v1.21 (Clement et al. 2000) and GeoDis v2.5 (Posada et al. 2000). Using this program, the statistical parsimony network was constructed for a 95% probability of parsimonious connection limit to infer the phylogenetic relationships among mitochondrial DNA haplotypes of the harbour porpoise. The ANeCA v1.2 program cannot break loops in the network from TCS, but deals with ambiguities by including them into clades during the nesting process. However, the ambiguities reduced the statistical power for detecting phylogeographic patterns. Thus, ambiguities in the haplotype network obtained were resolved as much as possible by coalescence theory (Crandall and Templeton 1993) using frequency criterion, topological criterion and geographical criterion by hand. The parsimony network with loops broken was then defined as a series of nested clades following proposed nesting rules (Templeton and Sing 1993; Templeton 2004). This nested cladogram along with actual geographic distance between haplotypes was used to produce the four statistics; $Dc(x)$, the average distance for all members of clade x from their geographical centre, $Dn(x)$, the average distance for all members of clade x from the geographical centre of the nesting clade y, $I\text{-}Tc(y)$, the average difference between interior and tip Dc values within the nesting clade y, and $I\text{-}Tn(y)$, the average difference between interior and tip Dn values within the nesting clade y, where x and y refer to a specific n-step and $n + 1$ step clade, respectively. The null hypothesis of no geographic association among haplotypes or clades at each nesting level was tested with these statistics by 10,000 random permutations of clades against sampling locality using the above-mentioned GeoDis program. The inference key given by Templeton (2004) was used to infer the historical process of the population at each nesting level.

Geographical information of the habitable area for the species but no samples was also needed to detect the sampling inadequacies in NCPA. The central Aleutian Islands and the Bering Sea were specified as the unsampled habitat.

Results

New mtDNA control region sequences (358 bps) of 58 harbour porpoises from Japanese waters and additional one from British Columbia contained 23 variable nucleotide sites, which defined 19 haplotypes. Ten of these haplotypes were previously unknown for the harbour porpoises in the North Pacific. These novel sequences were deposited in GenBank with accession numbers (AB499064–AB499073; see also supplementary material). Combining these 59 new sequences with those reported previously (Rosel et al. 1995; Chivers et al. 2002), a total of 93 variable sites were identified in 389 sequences, thereby defining 78 haplotypes in the harbour porpoises examined herein.

Genetic population structure of the North Pacific harbour porpoise

Pairwise φ_{ST} and F_{ST} estimates suggested essentially similar differentiation pattern of populations, with distinct differentiation between an east/south (Monterey—British Columbia) and a north/northwest group (Strait of Georgia and Alaska—Japan), although the F_{ST} values were mostly smaller than the φ_{ST} values (Table 1). AMOVAs confirmed the distinct structure between the east/south and north/northwest groups as mentioned earlier, with 11.87% of the total variance ascribed to differences between these two groups (Table 2, Analysis I, $P < 0.01$), while the variation within groups and strata were also statistically significant with 2.01% and 86.12% of the total variance (within groups: $P < 0.05$, within strata: $P < 0.001$, Analysis I), respectively. Additionally, mild genetic structures were suggested within the east/south group (Analysis II, 2.01% of the variance, $P < 0.05$) and within the north/northwest group (Analysis III, 2.25% of the variance, $P < 0.05$). Haplotype (h) and nucleotide diversities (π) for each stratum and group were higher in the east/south than the north/northwest group, and the h in the Strait of Georgia with a certain level of sample size was the lowest among the regions examined (Table 3).

The Mantel test showed a weak but significantly positive relationship between the genetic and geographical distances (Fig. 2, $R^2 = 0.27$, $P < 0.001$), suggesting an influence of isolation by distance with restricted gene flow in the population structure of the North Pacific harbour porpoises examined.

Phylogeography of the North Pacific harbour porpoise

The TCS program inferred 8 mutational steps at 95% connection limit, and the most plausible cladogram is presented with four nested levels in Fig. 3. However, haplotype 79 in Vancouver Island, haplotype 87 in British Columbia and haplotype 91 in San Francisco were separated from main clade with more than 9 mutational steps (not shown), and the NCPA did not infer any historical events in these clades.

The nested contingency analysis showed significant geographical association of some clades at all nested levels (Fig. 3; see also supplementary material). Among these, the estimate of Dc for a 3-step clade 3–4 with most haplotypes occurring in the Pacific Rim indicated that some tip clades were significantly less widespread than randomize

Table 1 Pairwise φ_{ST} and conventional F_{ST} estimates among the sampling strata based on 10,000 permutations

	MO	SF	OR	CR	WA	VC	SJI	BC	SG	CP	JP
MO		0.030***	0.069***	0.047***	0.037***	0.047***	0.046***	0.028***	0.189***	0.054***	0.073***
SF	0.017***		0.064***	0.002***	0.004***	0.039***	0.013***	0.023***	0.242***	0.039***	0.068***
OR	0.033***	0.075***		0.038***	0.046***	−0.019***	0.058***	0.002***	0.049***	0.008***	−0.002***
CR	0.003***	0.004***	0.004***		0.006***	0.016***	0.005***	0.016***	0.198***	0.023***	0.054***
WA	0.008***	0.032***	0.014***	−0.021***		0.025***	0.018***	0.019***	0.159***	0.031***	0.048***
VC	0.056***	0.040***	−0.010***	0.029***	0.046***		0.025***	−0.016***	0.083***	<0.001***	0.001***
SJI	0.030***	0.070***	−0.020***	0.002***	0.009***	0.012***		0.023***	0.208***	0.045***	0.076***
BC	0.041***	0.086***	−0.026***	0.025***	0.038***	−0.006***	0.001***		0.125***	−0.004***	0.008***
SG	0.109***	0.181***	−0.011***	0.095***	0.082***	0.018***	0.034***	−0.008***		0.111***	0.061***
CP	0.191***	0.279***	0.078***	0.204***	0.169***	0.082***	0.124***	0.039***	0.021***		0.005***
JP	0.237***	0.366***	0.099***	0.246***	0.195***	0.128***	0.153***	0.083***	0.029***	0.016***	

Below and above diagonal show the pairwise φ_{ST} and conventional F_{ST} estimates, respectively. Sample strata ID can be found in Fig. 1

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Table 2 The results of AMOVAs

	df	Variance components	Percentage variation	*P* value	Fixation index (φ_{ST})
Analysis I					
Among groups	1	0.345	**11.87**	**<0.01**	**0.14**
Among strata within groups	9	0.058	**2.01**	**<0.05**	**0.02**
Within strata	364	2.499	**86.12**	**<0.001**	**0.14**
Total	374	2.902			
Analysis II					
Among strata within groups	7	0.063	**2.01**	**<0.05**	**0.02**
Within strata	260	3.070	97.99		
Total	267	3.133			
Analysis III					
Among strata within groups	2	0.025	**2.25**	**<0.05**	**0.02**
Within strata	104	1.074	97.75		
Total	106	1.099			

The sample strata are grouped into east/south (Monterey—British Columbia) and north/northwest (Strait of Georgia, Alaska and Japan) in Analysis I. Analyses II and III show the results within east/south and north/northwest groups, respectively. Bold text indicates statistically significant variance partition and related φ_{ST} estimate

Table 3 Haplotype and nucleotide diversities and the results of selective neutrality tests for each stratum, the sample groups and entire North Pacific

Sample strata	No. of samples	*h*	π	Tajima's *D*	Fu's F_S	R_2
MO	58	0.86 ± 0.03	0.016 ± 0.009	0.30	−0.23	0.10
SF	17	0.96 ± 0.03	0.025 ± 0.013	−0.90	−1.45	0.14
OR	17	0.73 ± 0.11	0.012 ± 0.007	−0.81	−0.27	0.14
CR	20	0.91 ± 0.05	0.017 ± 0.010	−0.60	−1.83	0.13
WA	83	0.89 ± 0.02	0.014 ± 0.008	−0.53	−3.06	0.09
VC	18	0.81 ± 0.09	0.022 ± 0.012	−1.38	0.15	0.14
SJI	36	0.92 ± 0.02	0.014 ± 0.008	0.07	−2.47	0.11
BC	19	0.87 ± 0.07	0.018 ± 0.010	**−1.54**	−2.01	**0.13**
SG	24	0.38 ± 0.12	0.007 ± 0.004	−1.14	1.57	**0.13**
CP	25	0.83 ± 0.07	0.005 ± 0.003	**−2.14**	**−6.82**	0.13
JP	58	0.76 ± 0.06	0.005 ± 0.003	**−1.95**	**−12.57**	**0.11**
Sample groups						
East/south	268	0.90 ± 0.01	0.016 ± 0.009	**−1.68**	**−24.78**	**0.08**
North/northwest	107	0.71 ± 0.05	0.006 ± 0.004	**−2.20**	**−23.88**	**0.09**
Entire North Pacific	375	0.86 ± 0.02	0.014 ± 0.008	**−1.84**	**−24.88**	**0.07**

The bold text indicates the statistical significance at $\alpha = 0.05$, but $\alpha = 0.02$ for Fu's F_S. Sample strata ID can be found in Fig. 1

data. Additionally, the estimate of *Dn* indicated some tip clades with significantly large or small distance, and an interior clade 2–11 with significantly large distance from other clades within the clade 3–4. This suggested primarily restricted gene flow with ambiguous inference for a dispersal pattern, which also was inferred for a 1-step clade 1–16, probably because of inadequate sampling design. The estimate of *Dc*, *Dn, I-Tc* and *I-Tn* for a 3-step clade 3–1, 2-step clades 2–1 and 2–13, and 1-step clades 1–6 and 1–25 inferred isolation by distance pattern with restricted gene flow, which indicated characteristic pattern in that interior clade was significantly more widespread and/or tip clade was significantly less widespread than randomized data. The contiguous range expansion was inferred from the estimate of *Dc* for a 3-step clade 3–3 and 1-step clade 1–11, indicating that the interior clade was significantly less widespread and/or tip clade was significantly more widespread than randomize data. In addition, the estimate of *Dn* for the clade 3–3 indicated interior clades 2–1 and 2–14 with significantly small and large geographical distance from other clades within the clade 3–3, respectively. The estimate of *Dn* for a 2-step clade 2–7 indicated a tip clade 1–10 and an interior clade 1–11 with significantly large and small distance from other clades within the clade

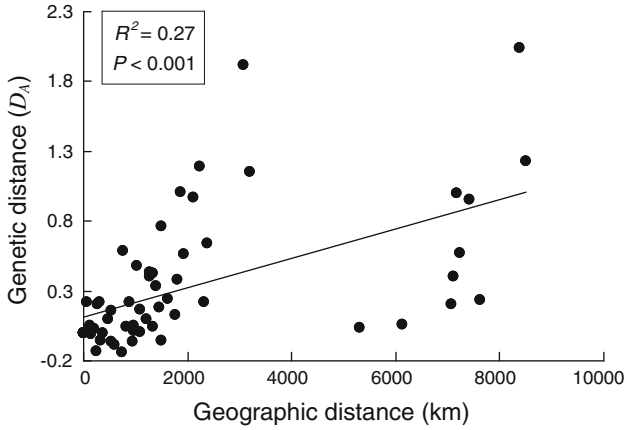

Fig. 2 Mantel test for the correlation between the genetic (D_A) and geographic distances (km) in harbour porpoises in the entire North Pacific

2–7, respectively. Additionally, the estimate of *I-Tn* for the clade 2–7 showed significantly small value, suggesting allopatric fragmentation.

Historical demography of the North Pacific harbour porpoise

The goodness-of-fit test for the observed mismatch distributions in the harbour porpoise did not deviate significantly from the simulated distribution under the sudden expansion model in entire North Pacific (Fig. 4a, $P = 0.61$), as well as east/south (Fig. 4b, $P = 0.35$) and north/northwest groups (Fig. 4c, $P = 0.42$), although the distribution curve in the entire North Pacific and east/south group were obviously bimodal. Significantly negative Tajima's D and Fu's F_S, and small R_2, also supported a sudden expansion in the entire North Pacific, east/south and north/northwest groups (Table 3), however, suggest a rather complex historical pattern of population growth, marginally pertaining to the sudden expansion model. Such demographic pattern was also inferred from non-significant D, F_S and R_2 estimates in almost all strata of the east/south group.

Population expansion time of the harbour porpoise in the entire North Pacific was estimated with the τ to be approximately 300,000–400,000 years ago (Table 4), whereas it was approximately 300,000–400,000 and 40,000–50,000 years ago in the east/south and north/northwest groups, respectively.

Discussion

The present mtDNA analysis of the North Pacific harbour porpoise demonstrated: (i) substantial genetic divergence between the north/northwest (Strait of Georgia, Alaska and Japan) and east/south (Monterey–British Columbia) groups, (ii) higher genetic variation in the east/south than the north/northwest group, (iii) distribution influenced by contiguous range expansion with restricted gene flow and (iv) demographic expansion influenced by the large-scale climate change in the late to middle Pleistocene.

The pairwise φ_{ST} estimates and AMOVAs clearly showed geographic structuring in the North Pacific harbour porpoise (Table 1), showing distinct genetic divergence between the east/south and north/northwest groups, with boundary near British Columbia, and weak to moderate divergence within the east/south and north/northwest groups (Table 2, Analyses II and III). The observed break around British Columbia may be partly compatible with the previous molecular studies, in that genetic differentiation was indistinct within the Northeast Pacific, but a certain level of differentiation was detected across the eastern North Pacific (Rosel et al. 1995; Chivers et al. 2002). Gaskin (1984) has reported previously the divergence between the eastern and western North Pacific using direct and indirect bycatch information. Amano and Miyazaki (1992) also found the morphological differences in the harbour porpoise between the eastern and western North Pacific. The present and previous molecular studies suggest that the estimated boundary, near British Columbia, between the western and eastern populations is further east than suggested by morphological study and capture information (Gaskin 1984; Amano and Miyazaki 1992).

The observed star-like genealogy of haplotypes from harbour porpoises in north/northwest group (e.g. clade 3–4 in Fig. 3) suggests a recent population growth from a few founders (Avise 2000), whereas the haplotype genealogy in the east/south group (clades 3–1, 3–2 and 2–1 in Fig. 3) implies rather complex population history, with increased genetic diversity as inferred from the h and/or π (Table 3). These findings suggest the older population history of the east/south group than the north/northwest group, and the dispersal of harbour porpoises from south to north/northwest in the North Pacific.

The five different approaches, i.e. the NCPA (Fig. 3), genetic diversities (Table 3), mismatch distribution analysis (Fig. 4 and Table 4), neutrality tests (Table 3) and Mantel test (Fig. 2), suggested contiguous range expansion from south to north/northwest along the Pacific Rim with restricted gene flow and/or rapid recovery from a bottleneck during the Pleistocene, about 300,000–400,000 years ago, with a second expansion in the north/northwest region 40,000–50,000 years ago. The NCPA has been criticized for its high frequency of false positives, particularly for the inference of restricted gene flow and contiguous range expansion (e.g. Knowles and Maddison 2002; Panchal and Beaumont 2007; Petit 2008).

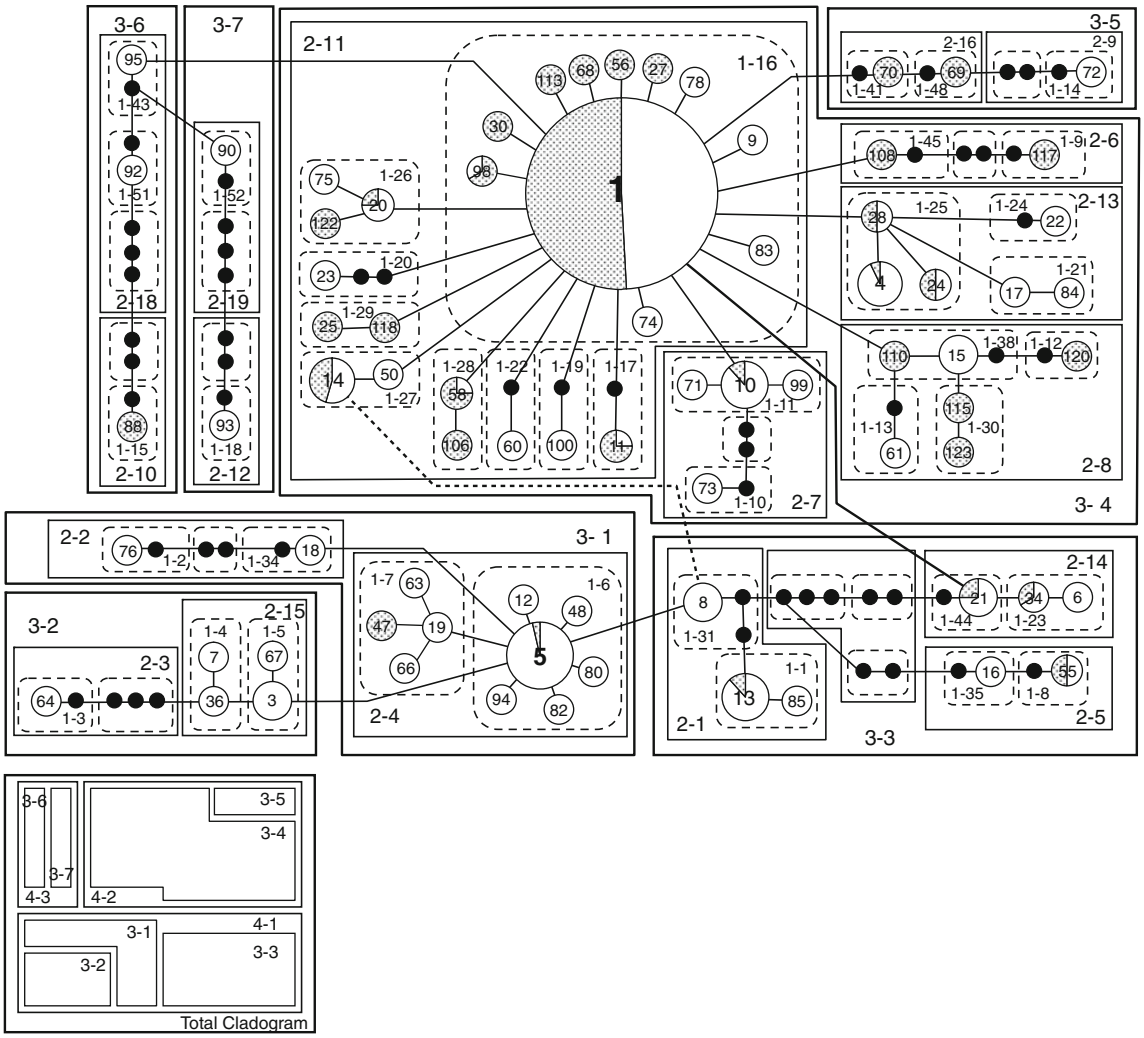

Fig. 3 Nested design for the mitochondrial DNA control region haplotypes of the harbour porpoise in the North Pacific. Each line indicates one mutational step. Reticulations which could not resolved by coalescence theory are shown by heavy dashed line. *Circles* represent haplotypes and the size refers to approximate haplotype abundance, with coloured strata groups (white: Monterey—British Columbia, half-tone dotted meshing: Strait of Georgia, Alaska and Japan). Small solid circles indicate intermediate haplotypes not found in this study

On the other hand, Templeton (2004, 2008) defended these criticisms based on the simulation artefacts and the discrepancies with real data in the simulations. Keeping these arguments in mind, the NCPA was used as one of multiple methods to examine phylogeographical patterns in the present study, and the obtained results were mostly compatible with those from other methods as mentioned earlier. Therefore, a discordant, single clade inference, such as allopatric fragmentation, was excluded from consideration herein.

The timing of the two above-mentioned demographic changes appears matched with the interglacial stages before the last glacial maximum (Rohling et al. 1998). Moreover, the observed pattern of haplotype distribution and genetic diversity, lower in the north than south, along the Pacific Rim also implies "leading edge expansion" in the North Pacific (Table 3). This pattern is thought to be a consequence of rapid post-glacial colonization by a population at the most northern refugium into virgin territory (Hewitt 2000). In this context, the lowest haplotype diversity in the Strait of Georgia among the examined strata, the genetic differentiation between the east/south and north/northwest groups, and the suggested break around British Columbia, in spite of the lack of geographic barrier, may favour a scenario of interglacial range expansion of the harbour porpoise to the Far East and subsequent glacial decrease or extinction of the populations around British Columbia. Cycling climatic fluctuation and associated glacier cycles could lead to the lowest haplotype diversity and the genetic break observed around British Columbia.

The occurrence of glacial refugia in the Pliocene and Pleistocene might also have influenced the genetic

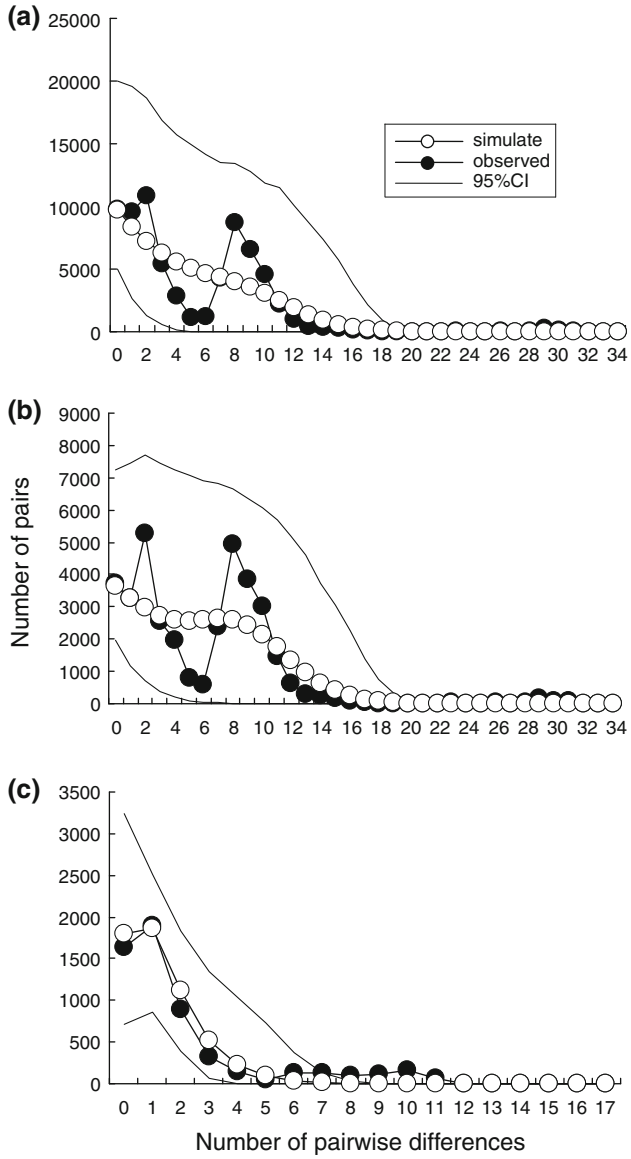

Fig. 4 Mismatch distribution under a sudden expansion model for the harbour porpoise in each sample group; **a** entire North Pacific, **b** east/south (Monterey–British Columbia) and **c** north/northwest (Strait of Georgia, Alaska and Japan)

structure of other organisms in glaciated regions (e.g. Harlin-Cognato et al. 2006). In fact, North Atlantic harbour porpoises have been suggested to be differentiated strongly between the east and west region, with the glacial refugia on either side of the Atlantic during the last glacial maxima (Rosel et al. 1999a; Tolley et al. 2001; Tolley and Rosel 2006). The break of genetic variation around British Columbia as observed in the present study also suggests two independent refugia in the North Pacific, as the coastal waters of British Columbia have had substantially less habitat for harbour porpoises because of a Cordilleran Ice Sheet during the early and middle Pleistocene in this region (Ryder et al. 1991). Ice-free regions in the Sea of Okhotsk, Aleutian Islands, Bering Sea, Gulf of Alaska and coastal North America between southeast Alaska and California were likely to provide glacial refugia in Pleistocene for the stellar sea lion *Eumetopias jubatus* and the harbour seal *Phoca vitulina*, both of whom have similar distribution and genetic structure to those of harbour porpoises in the North Pacific (Bickham et al. 1996; Westlake and O'Corry-crowe 2002; Harlin-Cognato et al. 2006). Considering these previous findings, the observed genetic break and the aforementioned haplotype genealogy, the Gulf of Alaska or the Bering Sea and southern part of the coastal North America might be refugia for harbour porpoises in the North Pacific.

An alternative explanation for the observed genetic structure includes oceanographic processes, such as ocean currents, salinity and temperature gradients, sea floor topology and other geographic features, which would provide opportunities for isolation and differentiation of marine organisms by limiting their dispersal (Jørgensen et al. 2005). In cetaceans having extremely high dispersal capability, however, the above-mentioned oceanographic features would not affect their distribution directly. Instead, these features may lead cetacean populations to differentiate indirectly by influencing the distribution and abundance of prey species (Kasuya et al. 1988; Fullard et al. 2000). For instance, sea surface temperature was a primary factor implicated in the genetic differentiation in some

Table 4 The parameters of the demographic expansion model for each sample group and entire North Pacific

	East/South			North/Northwest			Entire North Pacific		
	Mean	Low	Up	Mean	Low	Up	Mean	Low	Up.
τ	9.84	1.76	15.51	1.21	0.00	3.03	9.65	0.50	15.90
θ_0	0.23	0.00	2.90	0.06	0.00	0.40	0.19	0.00	2.59
θ_1	2664.85	4.78	∞	73984.61	4.40	∞	8153.53	3.26	∞
SSD			0.02			0.01			0.02
P value			0.42			0.35			0.61

τ time since expansion measured in mutational time units, θ_0 and θ_1 are the population sizes scaled by mutation rate before and after expansion, *SSD* sum of squared deviation in goodness-of-fit test with indicated *P* value, respectively

cetaceans because the oceanographic features were affecting their prey. (Kasuya et al. 1988; Fullard et al. 2000). Gene flow among coastal bottlenose dolphins in South Australia was also thought to be restricted by oceanographic and geographic features (Bilgmann et al. 2007). In the eastern North Pacific, the North Pacific Current bifurcates into the southward flowing California Current and the northward flowing Alaska Current off the coast of the Vancouver Island. Movement and foraging areas of the northern fur seal *Callorhinus ursinus* in the eastern North Pacific are affected by the North Pacific Drift, Alaska Gyre and California Current, in terms of reduction in energetic expenditures and optimization of foraging opportunities (Ream et al. 2005). The genetic differentiation observed between the east/south and north/northwest regions of the Pacific Rim for harbour porpoises may also be due to ocean currents effectively restricting animal movement.

A previous study on coastal dolphins distributed in cool temperate waters of the southern hemisphere also showed that the population history of coastal odontocetes is sensitive to the climate change (Pichler et al. 2001). In addition, coastal populations of Pacific white-sided dolphins *Lagenorhynchus obliquidens* show lower levels of genetic diversity than offshore populations in Japanese waters, a pattern ascribed to a bottleneck effect or population expansion caused by past climate changes (Hayano et al. 2004). In contrast, the Dall's porpoise *Phocoenoides dalli*, a related species to the harbour porpoise, but distributed more in offshore waters, was shown to have maintained a stable effective population size in the past (Mcmillan and Bermingham 1996). Given these findings and the phylogeographic pattern observed here for the examined harbour porpoises, it would appear that coastal cetacean species are affected more severely by the past climate change in spite of their extremely high dispersal capability. Hence, the genetic differentiation of the harbour porpoise in the North Pacific could be ascribed to fluctuation of the climate in the middle to late Pleistocene.

In conclusion, the present mtDNA analysis revealed distinct structuring of the North Pacific harbour porpoise between the east/south and north/northwest groups with the break near British Columbia and weak to moderate structuring within the east/south and north/northwest groups. The observed structure might be shaped by multiple range expansions with restricted gene flow in a direction from south towards north/northwest along the Pacific Rim in the middle and late Pleistocene as inferred from the phylogeographic analyses. The Cordilleran ice sheet would keep providing harbour porpoises a reduced habitat in coastal British Columbia during the glacial stage in the Pleistocene after their expansion to the Far East and may have resulted in substantially reduced genetic diversity and genetic break in British Columbia. In addition, it appears that the glacial refugia in the Pleistocene and oceanographic features like the North Pacific Current also likely affect the phylogeography of harbour porpoises in the North Pacific. Future multilocus analysis combining mtDNA and nuclear DNA, which allows tracing both parental demographic traits, may help understanding the finer evolutionary process in the North Pacific harbour porpoise.

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