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Population Differentiation in the Pacific White-sided Dolphin *Lagenorhynchus obliquidens* Inferred from Mitochondrial DNA and Microsatellite Analyses

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ABSTRACT—We investigated genetic diversity and differentiation of the Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) in Japanese coastal waters and offshore North Pacific by analyzing mitochondrial DNA and nuclear microsatellite variation. A total of 519 bp of the mitochondrial control region was sequenced and five microsatellite locus were genotyped for 59 individuals. A high level of haplotypic diversity ($h=96.1\%$), moderate level of nucleotide diversity ($\pi=1.65\%$) and average expected heterozygosity ($H_E=0.66-0.76$) were within an extent of those reported for other odontocetes. Consistent genetic difference between the samples from Japanese coastal Pacific-Sea of Japan and offshore North Pacific was indicated by analyses of molecular variance (AMOVAs) based on mtDNA and microsatellite variations, comparison of genetic variabilities, and geographical distributions of mtDNA haplotypes and microsatellite alleles. This result suggests that Pacific white-sided dolphins in each of the above two areas belong to different populations between which gene flow has been severely restricted. The low genetic diversity and mtDNA genealogy of the population in Japanese coastal waters suggest that it originated from a small population that colonized the Sea of Japan or that experienced population reduction when this Sea was isolated from the North Pacific during a glacial period in the Late Pleistocene.

Key words: *Lagenorhynchus obliquidens*, microsatellite, mitochondrial DNA, Pacific white-sided dolphin, population structure

INTRODUCTION

The Pacific white-sided dolphin (*Lagenorhynchus obliquidens* Gill, 1865) is one of the most abundant, widely distributed small cetacean species in the cool-temperate waters of the North Pacific Ocean (Brownell *et al.*, 1999). In the eastern North Pacific they are reported from the lower Gulf of California, Mexico (approximately 23°N) north to the northern Gulf of Alaska (approximately 61°N) (Leatherwood *et al.*, 1984; Walker *et al.*, 1986). In the western North Pacific, this species ranges from the East China Sea excluding coastal waters off Taiwan northward to the southern Sea of Okhotsk (Wang, 1993; Iwasaki, 1996). In Japanese

waters, this species occurs from northwest Kyusyu coast of the East China Sea north through the Sea of Japan to Hokkaido coast of the Sea of Okhotsk, and off the Pacific coast of the Kii Peninsula (approximately 33°N) northward along the Pacific coast of central and eastern Japan to the southern Kuril Islands (Fig. 1; Iwasaki, 1996). Although they have a more or less continuous distribution across the North Pacific between latitudes 38°N and 47°N, the density is apparently low in the offshore waters west of 150°E (Fig. 1; Hobbs and Jones, 1993; Miyashita, 1993).

Several populations of the Pacific white-sided dolphin have been recognized from the eastern North Pacific. Walker *et al.* (1986) reported that the animals off Baja California had consistently larger crania than those from northern California and suggested that the two morphotypes belong to different populations. Likewise Lux *et al.* (1997) found genetic subdivision, based on mitochondrial DNA control-region sequences, among samples from the coastal and offshore waters of the eastern North Pacific and suggested four populations: off Baja California; off California to Oregon;

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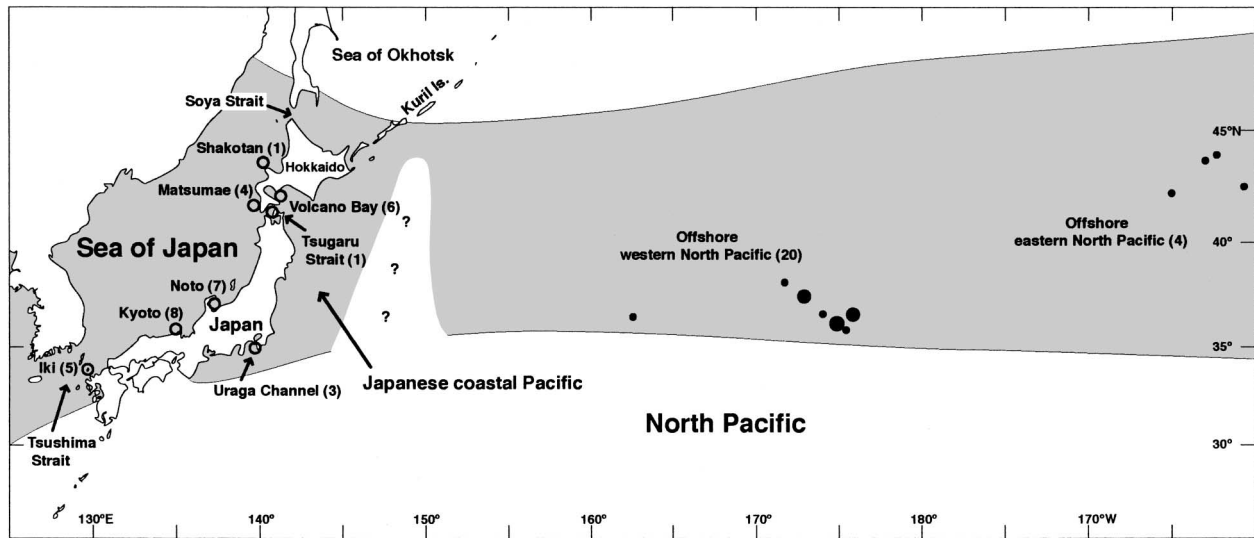


Fig. 1. Sampling localities of Pacific white-sided dolphins. Open and closed circles indicate Japanese coastal waters sample and offshore North Pacific sample, respectively. The size of the closed circles is proportional to the number of individuals collected. Sample size for each locality is shown in parenthesis. The distributional range of the Pacific white-sided dolphin is indicated by a gray area.

off British Columbia and Alaska and in the offshore waters east of 160°W.

In the western part of its range, there is a little information about population subdivisions. Sleptsov (1955) described specimens from the southeast Sea of Okhotsk as a new species, *Lagenorhynchus ognevi*, but Tomilin (1967) showed that the diagnostic features of *L. ognevi* all fell within the range of individual and age variation of *L. obliquidens* in the eastern North Pacific. Therefore *L. ognevi* has been regarded as a synonym of *L. obliquidens*. Although Miyazaki and Shikano (1997) probably overestimated their results due to sampling bias (see discussion in Iwasaki and Kasuya, 1997), they reported that specimens from Iki Island (Tsushima Strait) were larger in cranium and asymptotic body length than those from the offshore western North Pacific (158°–180°E). Geographical differences in growth pattern and asymptotic body length are suggested among Iki Island-Sea of Japan-Sea of Okhotsk, western North Pacific, and central North Pacific (approximately 170°E–145°W) (Iwasaki and Kasuya, 1997). These findings suggest that the species has at least one population in the coastal waters of Japan and other populations in the offshore waters of the North Pacific.

Little is known about seasonal migration and population structure of the Pacific white-sided dolphin within the waters around Japan. In the Sea of Japan, the dolphins were observed mostly in coastal waters off Iki Island in January–March, and off the central-northern Japan in June–July, suggesting they migrate northward along the coast of Japan in spring to off the western coast of Hokkaido and/or to the Sea of Okhotsk where they summer (Miyashita, 1986; Ohsumi, 1986). This suggestion is reasonable considering that the range of body lengths at cessation of growth for the northern Sea of Japan and Sea of Okhotsk sample reported by Miyazaki *et al.* (1991) is similar to the corresponding fig-

ure for the Iki Island sample in Takemura (1986). They have been also sighted in the Tsugaru Strait mainly in May–June, probably on the way from the Sea of Japan to the Pacific (Kawamura *et al.*, 1983). This migration pattern resembles that of the Sea of Japan-Okhotsk Sea population of Dall's porpoises (Miyashita and Kasuya, 1988; Amano and Kuramochi, 1992). Off the Pacific coast of Japan, white-sided dolphins occur north of the Kii Peninsula, and observed off the northern Japan in summer-autumn (Miyashita, 1993; Tsutsui *et al.*, 2001), but information on their migration is fragmentary and limited. Other biological information that infers population identity is also lacking. Therefore, whether white-sided dolphins off the Pacific coast of Japan constitute a discrete population or belong to the Sea of Japan and/or offshore North Pacific is not known.

In contrast to eastern North Pacific, no investigation using genetic markers had been carried out to examine genetic diversity or to identify population subdivision of Pacific white-sided dolphins in the western side of North Pacific. In the present study, we investigated genetic diversity and genetic differentiation in Pacific white-sided dolphins based on variations in the mtDNA control region and five nuclear microsatellite loci. We thereby discussed population structure and evolutionary process of population differentiation of the species in Japanese coastal waters and offshore North Pacific.

MATERIALS AND METHODS

Samples and DNA extraction

Tissue samples were collected from 59 Pacific white-sided dolphins (Fig. 1). Muscle, liver or skin was obtained from 42 dead animals and blood was collected from 17 live animals kept in aquaria. Animals taken by a scientific research cruise in the offshore western North Pacific in 1984 (n=20; Miyazaki and Fujise, 1985) and those incidentally caught by drift nets in the offshore eastern North Pacific

in 1991 ($n=4$) were treated as the offshore North Pacific sample. Animals taken by culling at Iki Island (Kasuya, 1985) in 1980 ($n=5$), those incidentally killed in coastal net fisheries such as trap nets or stranded on Japanese coast between 1991 and 2002 ($n=13$) and the animals incidentally caught in Japanese coastal net fisheries between 1983 and 1998 and transferred to aquaria ($n=17$) were treated as the sample of Japanese coastal waters.

Japanese coastal waters generally include parts of the Sea of Japan, Sea of Okhotsk and western North Pacific. In this study, however, we limited the term to Japanese coastal waters where our materials have been collected (i.e. eastern Sea of Japan and western North Pacific west of 150°E , Fig. 1). For studies of populations of such species as the Pacific white-sided dolphin which has vast distributional range and migrates seasonally, samples would be ideal if collected within a certain year and a certain season. However, we used samples collected in several seasons over a period of 22 years, so we showed further information on each sample in Appendix for reference.

Tissue samples were preserved in 95% ethanol or stored frozen at -80°C . Total DNA was extracted following standard proteinase K digestion and phenol-chloroform extraction described in Hayano *et al.* (2003) or using a DNeasy® Tissue Kit (QIAGEN).

Mitochondrial control-region sequencing and analysis

The 5' end of the mitochondrial control region and flanking tRNAs were amplified using the polymerase chain reaction (PCR; Saiki *et al.*, 1988) with the primers L15926 (5'-ACACCAGTCTTG-TAAACC-3') modified from Kocher *et al.* (1989) and H00034 (5'-TACCAAATGTATGAAACCTCAG-3') from Rosel *et al.* (1994). The PCR was performed in a Perkin-Elmer thermocycler (model 2400) on a 50- μL scale. Each PCR reaction mixture contained 5–50 ng of genomic DNA, 15 mM Tris-HCl (pH 8.0), 75 mM KCl, 1.5 mM MgCl_2 , each dNTP at 150 μM , each primer at 0.3 μM and 1.25 units of *Taq* DNA polymerase (Takara Shuzo Co., Ltd.). The cycling profile consisted of an initial denaturation at 90°C for 2.5 min, followed by 35 cycles of 45 s at 94°C , 1 min at 46°C and 1.5 min at 72°C . The amplified products were purified using a QIAquick® PCR Purification Kit (QIAGEN) and subjected to cycle-sequencing reactions using an ABI PRISM™ Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's recommended conditions. All samples were sequenced in both directions with the primers used in the amplification. Sequences were analysed using an ABI 377 automated DNA sequencer, and the data were aligned by eye.

The amount and nature of DNA polymorphisms were assessed by estimating both nucleotide and haplotypic diversity (Nei, 1987). To test selective neutrality in the DNA region under examination, Tajima's (1989a) D -statistic was used. The calculations were carried out using the program package ARLEQUIN ver.2.000 (Schneider *et al.*, 2000). The neighbor-joining (NJ) method (Saitou and Nei, 1987) was applied to infer the phylogenetic relationships among mtDNA haplotypes from a pairwise matrix of the distance

based on Kimura's (1980) two-parameter model. The degrees of supports for internal branches were assessed by 1,000 bootstrap pseudoreplications (Felsenstein, 1985). The interpretation of bootstrap proportions (BPs) is still in a state of uncertainty (see Felsenstein and Kishino, 1993; Hillis and Bull, 1993). We tentatively followed Shaffer *et al.* (1997), and considered $\text{BPs} \geq 90\%$ as highly significant, $70 \leq \text{BPs} < 90\%$ as marginally significant, and $\text{BPs} < 70\%$ as constituting limited evidence of monophyly. The NJ analysis was performed using CLUSTAL W (Thompson *et al.*, 1994). A minimum spanning network of the mtDNA haplotypes was also constructed using the MINSNET (Excoffier and Smouse, 1994). An analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was conducted to measure the degree of genetic differentiation between geographical localities. The AMOVA calculates Φ_{ST} , analogous to Wright's (1965) F_{ST} , an index of population subdivision. The Φ_{ST} incorporates information on the genetic distance between haplotypes as well as the frequencies of haplotypes in each population. Genetic distances between pairwise haplotypes were calculated based on Kimura's two parameter model. The conventional F_{ST} was estimated by an analysis of variance of haplotypic frequencies with the AMOVA option. The significance of Φ_{ST} and F_{ST} was tested by multiple permutation (5,000 times) of the original data set.

Microsatellite genotyping and analysis

Pacific white-sided dolphin samples were genotyped using five microsatellite loci, namely EV001, EV037, EV094, EV096 and EV104 (Valsecchi and Amos, 1996). PCR amplifications were conducted in 10- μL reaction volumes containing 10–50 ng of genomic DNA, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl_2 , each dNTP at 150 μM , each primer at 0.3 μM and 1.25 units of *Taq* DNA polymerase (Takara Shuzo Co., Ltd.). The 5' end of one primer of each pair was labelled with a fluorescent phosphoramidite dye. The cycling profile consisted of an initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, locus-specific annealing temperature for 1 min and 72°C for 1 min, and was performed in a Perkin-Elmer thermocycler (model 2400). Annealing temperatures were: 48°C for EV104, 50°C for EV037 and EV096, 54°C for EV001 and EV094. Amplified products were mixed with a size standard (GeneScan 500 TAMRA, Applied Biosystems) and loaded onto an ABI 377 automated DNA sequencer. Allele fragments were scored according to fragment sizes calculated by GeneScan Analysis software (Applied Biosystems) and 2-bp increments among alleles of a dinucleotide repeat microsatellite locus.

Genetic diversity was assessed by the number of alleles per locus (A), observed (H_O) and expected heterozygosity (H_E). Tests of model assumptions for the microsatellite loci were performed in ARLEQUIN: linkage disequilibrium between pairs of loci were tested using a likelihood-ratio test (Slatkin and Excoffier, 1996) and deviations from Hardy-Weinberg equilibrium were examined for each locus in each locality using a probability test analogous to Fisher's exact test (Guo and Thompson, 1992). Heterogeneity of

Table 1. Genetic diversity estimates and Tajima's D -statistics on mtDNA control-region sequences (519 bp). An asterisk indicates significant deviation from beta-distribution at $P < 0.05$.

Locality	N	No. of haplotypes	Haplotype diversity h (%)	Nucleotide diversity π (%)	Mean pairwise differences	No. of polymorphic sites	Tajima's D -statistic
Japanese coastal waters ¹⁾	35	18	89.4	1.02	5.24	41	-1.70*
Offshore North Pacific	24	22	99.3	2.04	10.6	43	0.142
Total	59	38	96.1	1.65	8.57	60	-0.913

¹⁾ Sea of Japan and Pacific waters west of 150°E .

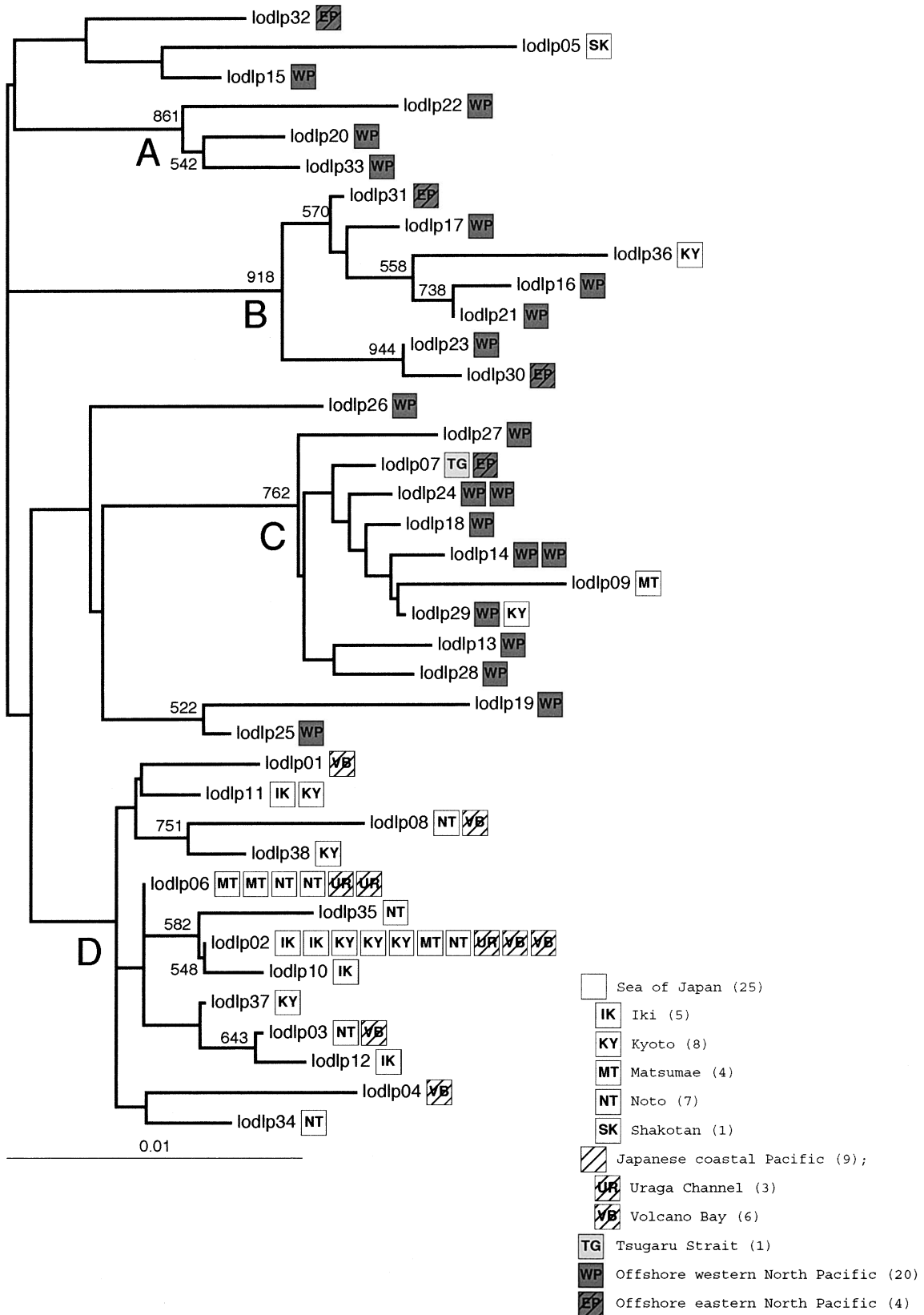


Fig. 2. Neighbor-joining dendrogram of 38 haplotypes. The numbers above the branch indicate bootstrap proportions (>50%) in 1000 replications. The squares indicate individuals bearing those haplotypes.

allele frequencies between localities was assessed by a similar probability test (Raymond and Rousset, 1995a) in GENEPOP (Raymond and Rousset, 1995b). Differentiation between localities was also quantified using two estimates calculated in analysis of variance of genotypic frequencies taking account of genetic distance between genotypes (AMOVA). F_{ST} is calculated using the number of different alleles as genetic distance based on the infinite allele model. R_{ST} is calculated using the sum of squared allele size difference based on the stepwise mutation model (Slatkin 1995). The significance of F_{ST} and R_{ST} was tested by multiple permutation (5,000 times) of the original data set.

RESULTS

Mitochondrial control-region sequences

Sequence analyses of 519 bases of the 5' end of the mtDNA control region revealed 60 variable sites defining 38 unique haplotypes among 59 individual dolphins (Table 1). The sequences of the 38 haplotypes have been deposited in GenBank under accession numbers AY625514–625551. As compared to most haplotypes consisting of 515 bp, lodlp15 had a 2-bp deletion, lodlp19 had a 4-bp insertion around site no. 100 and lodlp26 had a single bp deletion at site no. 2. These three haplotypes occur in the offshore western North Pacific sample alone.

Few reliable relationships among haplotypes were detected in an NJ dendrogram because of the many branches with low values of bootstrap proportion (BP < 70%, Fig. 2). However, three clusters which were supported with highly or marginally significant bootstrap proportions (node A: BP=86%, node B: BP=92%, node C: BP=76%) consisted of haplotypes from offshore western and eastern North Pacific except for four haplotypes (lodlp07, 09, 29 and 36). On the other hand, the samples from the Sea of Japan and Japanese coastal Pacific shared several haplotypes, and almost all haplotypes occurring in Japanese coastal waters constituted a single cluster though bootstrap support was low (node D: BP < 50%).

The number of haplotypes in Japanese coastal waters and offshore North Pacific were 18 and 22, respectively (Table 1). Only two haplotypes (lodlp07 and 29) were shared between these two samples (Fig. 2). For nucleotide diversity or mean pairwise differences, the estimates of the offshore North Pacific sample ($\pi=2.04\%$) were nearly double of those of Japanese coastal waters ($\pi=1.02\%$). A large negative Tajima's D -value was observed in the Japanese coastal waters sample ($D=-1.70$, $P=0.035$).

In a minimum spanning network, haplotypes from the Japanese coastal waters also formed a relatively compact

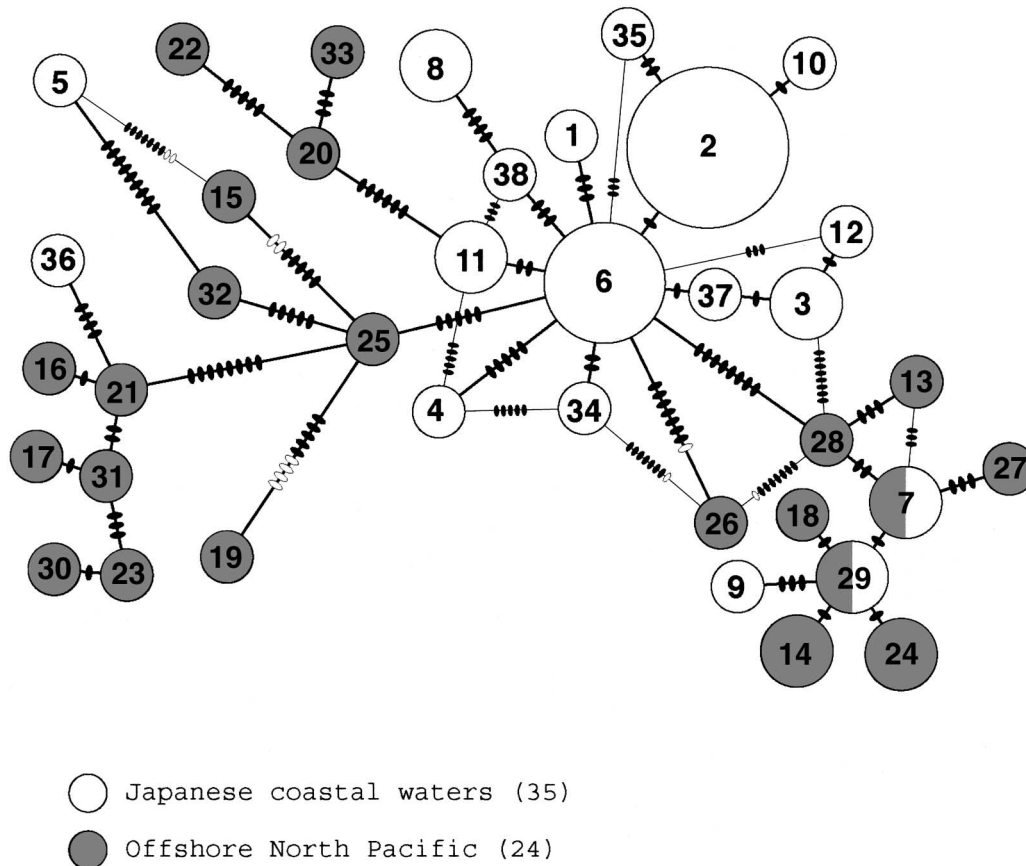


Fig. 3. Minimum-spanning network of the 38 haplotypes. The numerals inside circles indicate haplotype code numbers. The size of each circle is proportional to the number of individuals bearing that haplotype. Each circle is divided to represent the proportion of samples from each locality. Substitutions and indels are indicated by closed and open ovals, respectively. Alternative connections (thin lines) indicate homoplasy.

and reticulated cluster having haplotype lodlp06 at the center from which other haplotypes radiated (Fig. 3). Three clusters mainly consisting of the offshore North Pacific haplotypes were diversified around the cluster of Japanese coastal waters haplotypes, and those corresponded to the node A, B and C clusters in the NJ dendrogram.

The AMOVA results indicated genetic subdivision between two localities (Table 2). Significant ($P < 0.001$) F_{ST} and Φ_{ST} values were obtained between the offshore North Pacific and Japanese coastal waters samples. These results indicated substantial genetic differentiation between the Japanese coastal waters and the offshore North Pacific. Estimates of the number of female migrants per generation (Nm) between Japanese coastal waters and the offshore

North Pacific calculated from F_{ST} and Φ_{ST} were 8.51 and 1.50, respectively.

We roughly estimated the divergence time between the samples from two broader geographic regions, Japanese coastal waters and offshore North Pacific, based on a nucleotide divergence (d_A ; Nei, 1987) at the 519-bp control-region sequences of 0.454%. Using a nucleotide divergence between *L. obliquidens* ($n=59$, this study) and *L. obscurus* ($n=4$, GenBank AF113488–113491) of 4.43% and a divergence time (T) between these sister species of 0.74–1.05 million years (Hare *et al.*, 2002), we estimated the evolutionary rate (λ) at control region of *Lagenorhynchus* as 2.11–2.99% per site per million years by calculating $\lambda = d_A/2T$. Then we estimated the divergence time between the Japanese coastal waters and offshore North Pacific samples at $7.6\text{--}10.7 \times 10^4$ years ago.

Table 2. Nucleotide divergence and results of analyses of molecular variance (AMOVAs) on mtDNA control-region sequences and five microsatellite loci.

	Japanese coastal waters ¹⁾ / Offshore North Pacific	
Nucleotide divergence		
d_A (%)	0.454	
MtDNA		
F_{ST}	0.0555*	($Nm = 8.51$)
Φ_{ST}	0.250*	($Nm = 1.50$)
Microsatellite		
F_{ST}	0.0550*	($Nm = 4.30$)
R_{ST}	0.0768*	($Nm = 3.00$)

¹⁾ Sea of Japan and Pacific waters west of 150°E.
Permutation test: *, $P < 0.001$.

Microsatellite analysis

A total of 39 distinct alleles were observed at five loci. The total number of alleles at each locus ranged from three for locus EV001 to 11 for locus EV104.

No deviation from Hardy-Weinberg equilibrium was detected at five loci in two localities examined in this study. Significant linkage disequilibrium was not found between any pairwise loci. We therefore conclude that there was no evidence to reject the hypothesis that these loci provided independent information.

Summary statistics for microsatellite variation in each locality are shown in Table 3. For observed and expected heterozygosity averaged over loci, lower values were observed in the Japanese coastal waters sample ($H_O=0.64$,

Table 3. Summary of microsatellite results from Pacific white-sided dolphins. Number of individuals (N), range of allele sizes (R), number of alleles (A), number of private alleles (in parentheses), observed (H_O) and expected (H_E) heterozygosity.

Locality	Locus	EV001	EV037	EV094	EV096	EV104	Mean all loci
Japanese coastal waters ¹⁾	N	35	35	35	35	35	35
	R	118–122	187–195	244–260	190–208	148–166	
	A	3 (0)	5 (0)	5 (0)	9 (2)	8 (1)	6.0 (0.6)
	H_O	0.60	0.57	0.31	0.89	0.82	0.64
	H_E	0.56	0.68	0.37	0.85	0.83	0.66
Offshore North Pacific	N	24	24	24	24	24	24
	R	118–122	187–197	244–272	192–206	134–164	
	A	3 (0)	6 (1)	9 (4)	8 (1)	10 (3)	7.2 (1.8)
	H_O	0.58	0.79	0.88	0.92	0.83	0.80
	H_E	0.56	0.77	0.79	0.86	0.84	0.76
Total	N	59	59	59	59	59	59
	R	118–122	187–197	244–272	190–208	134–166	
	A	3	6	9	10	11	7.8
	H_O	0.59	0.66	0.54	0.90	0.83	0.71
	H_E	0.54	0.76	0.59	0.86	0.83	0.72

¹⁾ Sea of Japan and Pacific waters west of 150°E.

$H_E=0.66$) than in the offshore North Pacific sample ($H_O=0.80$, $H_E=0.76$). Likewise, mean number of total alleles, or private alleles, per locus for the Japanese coastal waters sample was smaller than for the offshore North Pacific. These lower diversity in the Japanese coastal sample resulted from low variability in locus EV037 and especially in locus EV094.

Exact tests for allele frequencies between Japanese coastal waters and the offshore North Pacific detected significant heterogeneity at locus EV037, EV094, and EV096 ($P<0.01$).

The AMOVA between the Japanese coastal waters and offshore North Pacific samples based on microsatellite variation indicated the same results as the AMOVA based on mtDNA variation (Table 2). Both F_{ST} and R_{ST} values between the Japanese coastal waters and offshore North Pacific samples were significant ($P<0.001$). Estimates of number of migrants (Nm) between the Japanese coastal waters and offshore North Pacific samples calculated from F_{ST} and R_{ST} were 4.30 and 3.00, respectively.

DISCUSSION

Genetic diversity and population structure

For mtDNA diversity, a high level of genetic variations in haplotypic diversity (overall $h=0.961$) and moderate variability in nucleotide diversity (overall $\pi=0.0165$) of Pacific white-sided dolphins are comparable to that of other odontocete species that are expected to have relatively large and stable population sizes (e.g., bottlenose dolphins (*Tursiops* spp.; Wang *et al.*, 1999), common dolphins (*Delphinus delphis*; Rosel *et al.*, 1994), striped dolphins (*Stenella coeruleoalba*; Archer, 1996), Dall's porpoises (*Phocoenoides dalli*; Hayano *et al.*, 2003) and harbor porpoises (*Phocoena phocoena*; Rosel *et al.*, 1995)). This result is reasonable considering that the Pacific white-sided dolphin is one of the most abundant cetaceans at present, with mean population estimates of 931,000 (Buckland *et al.*, 1993) or 988,000 (Miyashita, 1993) in the North Pacific Ocean and historical large population size of this species is suggested by intermixed genealogical patterns at nuclear loci observed between this and the sister species *L. obscurus* (Hare *et al.*, 2002).

The average H_E value at five microsatellite loci ranged from 0.66 to 0.76. As expected for these loci with high mutation rates, the values are substantially higher than the value estimated from allozyme data. Shimura and Numachi (1987) examined 19 allozyme loci in 30 Pacific white-sided dolphins from the coastal waters of Iki Island and found that six loci were polymorphic. The average H_E value of these six loci estimated from their data was 0.30. The mean H_E value estimated from microsatellite loci for Pacific white-sided dolphins was lower than that found in two species of Phocoenidae, but within the range of that found in other species of Delphinoidea using various microsatellite loci: a mean H_E value of 0.91 at five microsatellite loci in Dall's porpoises

(Hayano and Amano, unpublished data), 0.87 at six loci in harbor porpoises (Rosel *et al.*, 1999), 0.64 at eight loci in long-finned pilot whales (*Globicephala melas*; Fullard *et al.*, 2000), 0.65 at 15 loci in beluga whales (*Delphinapterus leucus*; Buchanan *et al.*, 1996), 0.76 at five loci in bottlenose dolphins (*Tursiops truncatus*; Shinohara *et al.*, 1997) and 0.76 at five loci in Indo-Pacific bottlenose dolphins (*T. aduncus*; Krützen *et al.*, 2001).

Consistent genetic difference between Pacific white-sided dolphins from the two broader geographic regions, Japanese coastal waters and offshore North Pacific, was indicated by results of AMOVA based on both mtDNA and microsatellite variations along with comparisons of genetic variabilities and distributions of mtDNA haplotypes and microsatellite alleles, while no geographical or seasonal signal was found within the two regions. This result is also consistent with differences of skull and body size between the dolphins from the coastal waters of Japan and offshore North Pacific found in the previous studies (Miyazaki *et al.*, 1991; Iwasaki and Kasuya, 1997; Miyazaki and Shikano, 1997). These genetic and morphological differences suggest that Pacific white-sided dolphins in Japanese coastal waters and offshore North Pacific belong to different demographic units and that dispersal and gene flow have been severely restricted between the two regions.

Miyashita (1993) reported that Pacific white-sided dolphins were scarcely sighted in the waters between 145°E and 150°E (Fig. 1). This discontinuous distribution of the species may reflect an actual boundary between the populations in Japanese coastal waters and offshore North Pacific. Although no physical barrier seems to exist in an oceanic environment, many odontocete populations have been found to segregate according to water masses, which have characteristic oceanographic traits. For example, Fullard *et al.* (2000) suggested that population structure of long-finned pilot whales in the North Atlantic correlated with sea surface temperature. In the western North Pacific, two local forms of short-finned pilot whales (*Globicephala macrorhynchus*) occur in the Kuroshio waters and in the Mixed Water Region between Fronts of the Kuroshio and the Oyashio currents, respectively (Kasuya *et al.*, 1988). Similarly, migration routes or breeding grounds of Dall's porpoises appear to correspond to population specific current regions or gyres (Kasuya and Ogi, 1987; Miyashita and Kasuya, 1988; Yoshioka *et al.*, 1990). Therefore it is a probable supposition that Pacific white-sided dolphins also do not migrate over a boundary of water masses.

Although the present study suggested that Pacific white-sided dolphins in the coastal waters of Japan and the offshore North Pacific belong to different populations, small sample size hinder the further consideration of population subdivision within the each region. In the offshore North Pacific, geographic signals in genetic variation could not be detected, but Iwasaki and Kasuya (1997) suggested more than one population based on the possible difference in asymptotic body length between the dolphins from central

area (177.1–183.8 cm for females and 183.8–184.9 cm for males; Iwasaki and Kasuya, 1997) and those from western area (mean=173.9 cm; Miyazaki and Shikano, 1997). As discussed later, population subdivision possibly exists within the coastal waters of Japan as in the case of the coastal waters of North America. Further genetical, morphological and ecological studies with larger sample size and information on correspondence between sampling localities and populations should be necessary for population identification within each region.

Genetic differentiation and population process in Japanese waters

The sample of Pacific white-sided dolphins from Japanese coastal waters showed lower levels of mtDNA and microsatellite diversity than the offshore North Pacific sample. Nucleotide diversity in the Japanese coastal waters sample ($\pi=1.02\%$) was particularly low, and half of that estimated for the offshore North Pacific sample in this study ($\pi=2.04\%$) or that for populations in the eastern North Pacific ($\pi=2.11\%$; Lux *et al.*, 1997). In addition, few common haplotypes and a certain degree of lineage sorting of mtDNA haplotypes were observed between Japanese coastal waters and the offshore North Pacific, in contrast with no correlation between haplotype genealogy and geographical area detected by Lux *et al.* (1997). These distinctions of the dolphins in Japanese coastal waters suggest that the population in Japanese coastal waters has experienced a peculiar process of genetic drift, probably with strong gene flow barriers. Possible historical changes in the demography of the Japanese coastal waters population are inferred from another characteristic of mtDNA variation. A large negative Tajima's *D*-value was obtained for the Japanese coastal waters sample, and Tajima's test of selective neutrality indicated that the mean number of pairwise nucleotide substitutions was significantly small for the number of segregating sites in the sample. This suggests population disequilibrium caused by a past bottleneck or population expansion (Tajima, 1989b, 1993). Strong genetic drift probably due to small population size in the past is also inferred from the star-like phylogeny around the core and frequent haplotype in the Japanese coastal waters sample, which present a striking contrast to the deeply branching network with several distinctive lineages in the offshore North Pacific sample. Average heterozygosity is known to have a positive correlation with effective population size (Nei, 1983; Nei and Graur, 1984) and to be affected by a bottleneck event for subsequently long time (Nei *et al.*, 1975). The lower values of average heterozygosity and haplotypic diversity observed in the Japanese coastal waters sample may also be remains of the past bottleneck effect, even though these values may suggest smaller abundance of the population.

Estimates of the time of divergence between the Japanese coastal waters and the offshore North Pacific samples ranged from 76,000 to 107,000 years ago. Considering the peculiar process of genetic drift in the Japanese coastal

waters population, these values would be overestimation (see Chakraborty and Nei, 1977; Gaggiotti and Excoffier, 2000). However, we consider that environmental or geological events in the Late Pleistocene (10,000–130,000 years ago) would have affected the process of population differentiation and the demography of the Pacific white-sided dolphins in Japanese coastal waters. Currently, the Sea of Japan is connected with the Sea of Okhotsk, the Pacific and the East China Sea through the Soya, the Tsugaru and the Tsushima Straits, respectively (Fig. 1). These straits are considered to have been open during the Riss-Würm interglacial period in the Late Pleistocene (Ohshima, 1990). However, subsequent sea level lowering in Würm glacial caused a landbridge connection at the Soya Strait and the Sea of Japan was completely isolated from the Sea of Okhotsk (Ono, 1990). Although it has been controversial whether the Tsugaru and Tsushima Straits, which are much deeper than the Soya Strait, were closed completely or not in the Würm glacial (Ohshima, 1990; Oba *et al.*, 1991; Keigwin and Grobarenko, 1992), migrations of marine organisms between the Sea of Japan and the Pacific or the East China Sea must have been severely restricted during this period. From this information and the above suggestions of the process of genetic drift, we infer that the population of the Pacific white-sided dolphins in Japanese coastal waters originated from a small founding population that colonized the Sea of Japan in an interglacial, or that the population was isolated from other populations in the North Pacific while the Sea of Japan was practically disconnected from the North Pacific due to the lowered sea level in a glacial period and then experienced a population reduction because of some environmental changes in the Late Pleistocene. The similar process of genetic drift and population history was also suggested in the Sea of Japan-Okhotsk population of Dall's porpoises (Hayano *et al.*, 2003).

Considering the genetic homogeneity of the Japanese coastal waters samples and the differentiation process of the Japanese coastal population suggested above, the present distribution of the Pacific white-sided dolphins may have extended from the Sea of Japan to the waters off the Pacific and Okhotsk coast of Japan quite recently, after the Sea of Japan connected with the North Pacific and the Sea of Okhotsk again.

The samples from the Volcano Bay and the Sea of Japan had common or closely related haplotypes of mtDNA, which suggests that the dolphins sighted off the Pacific coast of northern Japan in summer–autumn migrate from the Sea of Japan. This suggestion is consistent with the sightings of the dolphins in the Tsugaru Strait in May–June (Kawamura *et al.*, 1983).

However, it is unknown whether the dolphins observed off the Pacific coast of southern Japan in autumn–winter migrate from the Sea of Japan. Therefore, two different interpretations on the migration and population subdivision in the Japanese coastal waters are still possible; the dolphins belong to one large population and have several

migration patterns according to age classes or reproductive conditions, as segregative migration found in the Sea of Japan-Okhotsk population of the Dall's porpoise (Amano and Kuramochi, 1992), or another population has been differentiated in the Pacific coast of Japan from the population in the Sea of Japan though genetic distinction between these population is undetectable because of a certain level of gene flow and/or a recent divergence. Further genetical and morphological investigations on a finer scale with larger sample size, along with ecological research on seasonal migration and distributional patterns should be necessary for understanding the population structure of the Pacific white-sided dolphin in Japanese coastal waters.

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Appendix. MtDNA haplotype, collection date and locality for DNA samples.

Sample No.	Sex ¹⁾	Haplotype	Date	Locality
Japanese coastal waters (Sea of Japan and Pacific waters west of 150°E) N=35				
Lo-019	M	lodlp10	Jan-Mar 1980	Iki
Lo-020	M	lodlp02	Jan-Mar 1980	Iki
Lo-021	M	lodlp11	Jan-Mar 1980	Iki
Lo-022	M	lodlp12	Jan-Mar 1980	Iki
Lo-023	F	lodlp02	Jan-Mar 1980	Iki
Lo-017	F	lodlp02	11/08/1994	Kyoto
Lo-018	M	lodlp02	11/08/1994	Kyoto
Lo-056	F	lodlp02	05/25/1990	Kyoto
Lo-057	M	lodlp36	02/13/1992	Kyoto
Lo-058	M	lodlp11	10/21/1993	Kyoto
Lo-059	M	lodlp29	05/25/1990	Kyoto
Lo-060	F	lodlp37	04/17/1992	Kyoto
Lo-061	F	lodlp38	05/21/1991	Kyoto
Lo-048	F	lodlp03	04/30/1991	Noto
Lo-049	M	lodlp34	02/19/1996	Noto
Lo-050	F	lodlp06	02/19/1996	Noto
Lo-051	M	lodlp02	03/06/1998	Noto
Lo-052	F	lodlp35	03/09/1998	Noto
Lo-053	M	lodlp08	03/24/1998	Noto
Lo-054	F	lodlp06	03/24/1998	Noto
Lo-011	F	lodlp06	05/28/1998	Matsumae
Lo-012	M	lodlp02	05/28/1998	Matsumae
Lo-013	F	lodlp09	05/28/1998	Matsumae
Lo-014	F	lodlp06	05/28/1998	Matsumae
Lo-007	M	lodlp05	05/27/1998	Shakotan
Lo-009	U	lodlp07	06/03/1999	Tsugaru Strait
Lo-001	U	lodlp01	07/13/1999	Volcano Bay
Lo-003	U	lodlp02	07/13/1999	Volcano Bay
Lo-004	U	lodlp03	08/01/1999	Volcano Bay
Lo-005	M	lodlp04	07/01/1998	Volcano Bay
Lo-006	M	lodlp02	07/28/1998	Volcano Bay
Lo-010	F	lodlp08	07/04/1999	Volcano Bay
Lo-015	M	lodlp06	05/05/1983	Uraga Channel
Lo-016	M	lodlp06	05/05/1983	Uraga Channel
Lo-055	F	lodlp02	12/22/2002	Uraga Channel
Offshore North Pacific N=24				
Lo-024	M	lodlp13	05/23/1984	offshore western North Pacific
Lo-025	M	lodlp14	06/02/1984	offshore western North Pacific
Lo-026	F	lodlp15	06/02/1984	offshore western North Pacific
Lo-027	M	lodlp16	06/02/1984	offshore western North Pacific
Lo-028	M	lodlp17	06/02/1984	offshore western North Pacific
Lo-029	M	lodlp18	06/02/1984	offshore western North Pacific
Lo-030	M	lodlp19	06/03/1984	offshore western North Pacific
Lo-031	M	lodlp20	06/03/1984	offshore western North Pacific
Lo-032	M	lodlp21	06/03/1984	offshore western North Pacific
Lo-033	F	lodlp22	06/03/1984	offshore western North Pacific
Lo-034	M	lodlp23	06/03/1984	offshore western North Pacific
Lo-035	M	lodlp24	06/03/1984	offshore western North Pacific
Lo-036	F	lodlp25	06/03/1984	offshore western North Pacific
Lo-037	F	lodlp24	06/04/1984	offshore western North Pacific
Lo-038	M	lodlp26	06/04/1984	offshore western North Pacific
Lo-039	F	lodlp27	06/04/1984	offshore western North Pacific
Lo-040	M	lodlp28	06/04/1984	offshore western North Pacific
Lo-041	M	lodlp29	06/05/1984	offshore western North Pacific
Lo-046	M	lodlp14	06/03/1984	offshore western North Pacific
Lo-047	M	lodlp33	06/04/1984	offshore western North Pacific
Lo-042	F	lodlp30	08/02/1991	offshore eastern North Pacific
Lo-043	F	lodlp07	07/07/1991	offshore eastern North Pacific
Lo-044	M	lodlp31	07/05/1991	offshore eastern North Pacific
Lo-045	M	lodlp32	08/21/1991	offshore eastern North Pacific

¹⁾F, female; M, male; U, sex unknown.