

Preliminary mtDNA analysis of gray whales from Japan and Russia

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ABSTRACT

Gray whales (*Eschrichtius robustus*) are divided into western and eastern populations. This study is the first to present the genetic data obtained from the gray whales migrating to or from the breeding ground of the western population along the Japanese coast. We conducted mitochondrial DNA analysis on the samples of gray whales from Japan (western, N=6) and Russia (eastern, N=7) and analyzed the generated data in comparison to those of Leduc *et al.* (2002) and Lang *et al.* (2004) to better understand the genetic characteristics of these whales at the wider geographic area. The Japanese gray whales were those either stranded on beach or bycaught on set net along the Japanese coast from 1995 to 2007, and the Russian gray whales were those legally caught during the Chukotka aboriginal subsistence whaling in 2008. All of the mtDNA haplotypes found in the Japanese (five) and Russian (six) samples matched to some of the previously reported haplotypes. The level of genetic diversity of these samples described as haplotype diversity and nucleotide diversity were surprisingly high, suggesting either gene flow between the western and eastern populations or retention of ancient polymorphism without gene flow. No statistically significant difference in haplotype frequencies was detected between the JPN and RUS samples possibly due to the small sample sizes. The phylogenetic analysis of the mtDNA haplotypes found in this study and the past studies detected no distinct cluster for the Japanese whales, supporting the past observation that the western and eastern gray whales were indistinguishable at the evolutionary time scale.

KEYWORDS: GRAY WHALES, PACIFIC OCEAN, GENETICS

INTRODUCTION

Gray whales (*Eschrichtius robustus*) distribute along the North Pacific coast from Asia and Russia to United States and Mexico and are divided into the western (Okhotsk-Korea) and eastern (Chukotka-California) populations (Rice, 1998). The eastern gray whales breed in the Baja California waters in winter and feed in the Bering and Chukchi Seas during summer, while the western gray whales breed in the coastal waters near China and feed in the Okhotsk Sea off Sakhalin Island. After severe reduction of the population size during the commercial whaling period, the eastern population recovered its abundance to near pre-exploitation level but the western one has been believed to still remain small

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(IWC, 1998). Conservation and management of this species has been of concern for the range countries.

Identification of population structure is essential for effective management. Past genetic studies based on the genetic variations at mitochondrial DNA (mtDNA) and microsatellites demonstrated that the western and eastern populations were genetically distinct at the population level but not at the evolutionary level (Leduc *et al.*, 2002; Lang *et al.*, 2004). The population structure of the species hasn't been fully resolved yet, however, partly because the sample of what they call the western population in these past studies limited to the individuals from the narrow part of feeding ground off Sakhalin Island. These whales could have been the mixture of whales from both populations (e.g., Lang *et al.*, 2004) or could have come from the eastern population (e.g., Ilyashenko, 2009). Lang *et al.* (2004) found high genetic diversity within the samples and the differences in the level of genetic differentiation between males and females, that made the authors suspect extended migration of some eastern gray whales, especially males, to the area off Sakhalin Island. Ilyashenko (2009) proposed that the current gray whale population migrating to the area off Sakhalin Island was originated from the eastern gray whales recolonized after the extinction of the species in this area during the commercial whaling period. In order to address this issue and to conduct effective management of gray whales, it is important to analyze gray whales obtained from the entire range of the species.

This study is the first to present the genetic data obtained from the gray whales migrating to or from the breeding ground of the western population along the Japanese coast (Fig. 1; Kato *et al.*, 2010). We conducted mtDNA analysis on the samples of gray whales from Japan (western, N=6) and Russia (eastern, N=7) and analyzed the generated data in comparison to those of Leduc *et al.* (2002) and Lang *et al.* (2004) to better understand the genetic characteristics of these whales at the wider geographic area.

MATERIALS AND METHODS

Samples

Three stranded gray whales on beach and three bycaught ones on set net along the Japanese coast from 1995 to 2007 were used for this study (JPN; Table 1). Detailed information on these six animals can be seen in Kato *et al.* (2010). Seven gray whales caught during the Chukotka aboriginal subsistence whaling in 2008 were also used (RUS; Table 1).

mtDNA analysis

Total DNA from each of the whales was extracted from 0.05 g of skin or muscle tissue using the protocol of Sambrook *et al.* (1989). Extracted DNA was stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The first half (486 bp) of the mtDNA control region was amplified through the polymerase chain reaction (PCR) using the following primer set: light-strand MT4 (Árnason *et al.*, 1993) and heavy-strand Dlp 5R (5'-CCATCgAgATgTCTTATTTAAggggAAC-3'). PCR products were purified by MicroSpin S-400HR columns (Pharmacia Biotech). Cycle sequencing was performed with the same primers, using BigDye terminator cycle sequence Kit (Applied Biosystems, 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 ABI3100™ Automated DNA Sequencer (Applied Biosystems, Inc) following the protocols of the manufacture. For each sample both strands were sequenced.

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Data analysis

Published mtDNA sequences in Leduc *et al.* (2002) were extracted from GenBank for haplotype designation and data comparison. The published data was generated from analyzing eastern gray whale sample (N=120) collected from the several locations along the coast from Chukotka to California and western gray whale sample (N=45) collected from the northeastern coast of Sakhalin Island. Because analyzed length of the sequence in our study was 37 bp shorter than that of Leduc *et al.* (2002), the haplotype G and O (hereafter, G/O), L and U (L/U), W and X (W/X) in the original paper became indistinguishable, reducing the total number of the haplotypes from 36 in the original paper to 33.

The number of haplotypes and haplotype diversity were calculated following Nei (1987). The nucleotide diversity (Nei, 1987: equation 10.5) and its standard error (se) for population sampling and stochastic processes were calculated from the pair-wise differences between the mtDNA sequences using the Kimura's 2- parameter adjustment (Kimura, 1980). The randomized chi-square test of independence (Roff and Bentzen, 1989) was used to investigate the level of genetic differentiation. In each test a total of 10,000 permutations of the original data were performed. The level of genetic differentiation expressed as H_{ST} was calculated based on Hudson *et al.* (1992).

Phylogenetic reconstruction of the mtDNA sequences was made using the neighbor-joining method implemented in the PHYLIP version 3.5c program (Felsenstein, 1993). Genetic distance among haplotypes were estimated using the program DNADIST of the PHYLIP based on Kimura's 2- parameter model (Kimura, 1980) with an observed transition:transversion ratio of 1:20. The obtained tree was visualized using TreeView PPC (Page, 1996).

RESULTS

On the basis of sequence variation at the 486 bp of control region, five different mtDNA haplotypes were found from the six Japanese gray whales, while six different haplotypes were found from the seven Russian gray whales (Table 2). These haplotypes of the JPNs matched to haplotypes A (N = 1), B (1), G/O (1), L/U (2), and Z (1) in Leduc *et al.* (2002), whereas the haplotypes of the RUSs matched to C (2), H (2), R (1), V (1), AE (1), so that total number of haplotypes found from the two studies was still 33. Haplotype diversity and nucleotide diversity within samples was 0.933 and 0.0185 (se = 0.0058) for the Japanese sample and 0.952 and 0.0163 (se = 0.0032) for the Russian sample, respectively. The level of genetic differentiation between the Japanese and Russian samples expressed as H_{ST} was 0.0316 which was not significantly different from 0 ($p=0.164$). Statistical test failed to detect statistically significant difference in the haplotype frequencies between the JPN and RUS ($p=0.211$). Because it was difficult to extract the exact number of haplotypes within the western and eastern population samples from Lang *et al.* (2004), no statistical comparison was conducted between the samples from this study and the past study.

The neighbour-joining tree was constructed for 33 haplotypes (Fig. 2). No geographically specific cluster was detected.

DISCUSSION

We analyzed the sample of the gray whales collected along the Japanese coast. Therefore, significance of this study is that these analyzed whales definitely came from the western population. We did not have

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to worry about that we might have collected the individuals from the eastern population that migrated to the feeding ground of the western population.

The results of this study were very same to those of the past genetic studies (Leduc *et al.*, 2002; Lang *et al.*, 2004): the genetic diversity within each of the two samples was high and the eastern and western populations were phylogenetically indistinguishable. As already presented by the past genetic studies, the level of genetic diversity within the western population was surprisingly high as compared with a typical small population. Four possible explanations can be raised. Firstly, individual(s) from the eastern population could have been wrongly sampled as the western population. Secondly, there may be gene flow between the two populations. Thirdly, because of the long life and historically large abundance, the western population may still retains considerable amount of genetic diversity after severe, but recent, population reduction. Finally, the population size of the western population may not be as small as it has been estimated (e.g., Cooke *et al.*, 2008). Among these, the first one is the most unlikely because our sample came from migratory corridor between the feeding and breeding grounds of the western population. The site fidelity of gray whales during the breeding migration has been believed strong. If all of the western gray whales feed at the area off Sakhalin Island and have been completely covered through photo-identification, the population size of the western population is indeed small as estimated (Cooke *et al.*, 2008) and thus the fourth reason raised above becomes unlikely. Among the remained two, it is hard to decide. Because of the small sample size as no statistically significant genetic differentiation was detected between the Japanese and Russian samples, it was difficult to reliably estimate the level of gene flow in this study. The population reduction during the commercial whaling period was quite severe biologically but might not have been genetically, allowing the western population to retain ancient diversity. Continued population monitoring and survey is important to estimate population size and describe migration pattern of gray whales.

Five of the six individuals in the Japanese sample had the different haplotypes and those haplotypes were widespread in the phylogenetic tree. Although two (L/U and Z) of the haplotypes found in the Japanese sample were referred as the eastern population types in Leduc *et al.* (2002), the analysis of the additional individuals in Lang *et al.* (2004) detected these two in the sample collected from the area off the Sakhalin Island. Contrary to the large differences in the numbers and frequencies of the different haplotypes within the samples from the same region among the different studies (i.g., Leduc *et al.*, 2002; Lang *et al.*, 2004; this study), the total number of the different haplotypes found from these studies did not changed as much. This indicated that the inference drawn from the phylogenetic analysis in this study should reflect gray whales' evolutionary history. Sharing of quite many haplotypes between the two populations and their positions in the phylogenetic tree indicated recent divergence of the populations within the species.

During the last year's scientific committee meeting (IWC, in press), population status of the gray whales feeding at the area off Sakhalin Island, whether the whales were the member of the eastern, western or mixture of both populations, was discussed (Brownell *et al.*, 2009; Ilyashenko, 2009). On the basis of the results from mtDNA and microsatellite analyses, Lang *et al.* (2004) showed that the Sakhalin gray whale sample was genetically different from the eastern gray whale sample but raised at the same time the possibility of extended migration of the eastern gray whales to the area off the Sakhalin Island.

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With increase of the sample size, the newly analyzed individuals in the Sakhalin sample tended to have the haplotypes originally found only in the eastern sample but not newly discovered (see Leduc *et al.*, 2002 and Lang *et al.*, 2004). This could reflect the level of the ancient polymorphism retained in the Sakhalin sample or this could indicate that the number of the eastern gray whales migrating to the area off Sakhalin Island is more than we have anticipated. Use of our Japanese gray whales as a reference base sample is suitable to address this kind of issue. However, it is unfortunate that its sample size is small and only mtDNA data is available at this moment. We are thus planning to analyze our samples with more than ten microsatellite markers that should overcome the reluctance for population structure analysis attributable to the small sample size.

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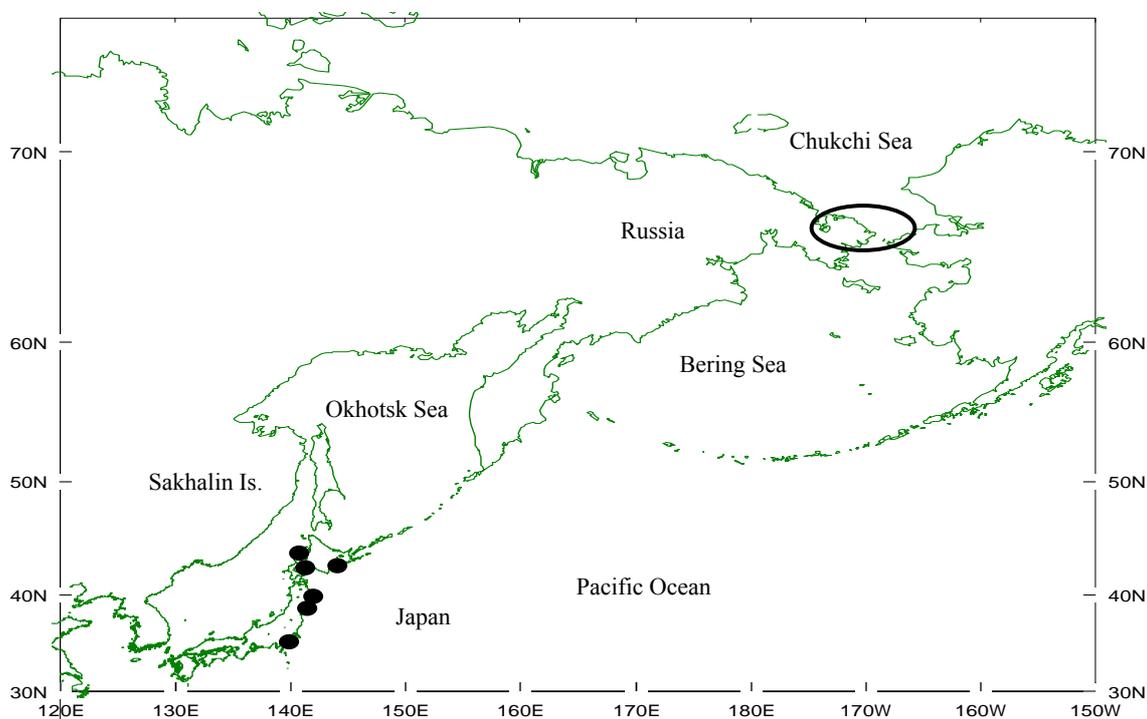


Fig. 1. Sample locations. Black dots indicate the sampling locations of the 6 Japanese gray whales, and open circle indicates the approximate area of the Chukotka aboriginal subsistence whaling collecting the 7 Russian gray whales for this study.

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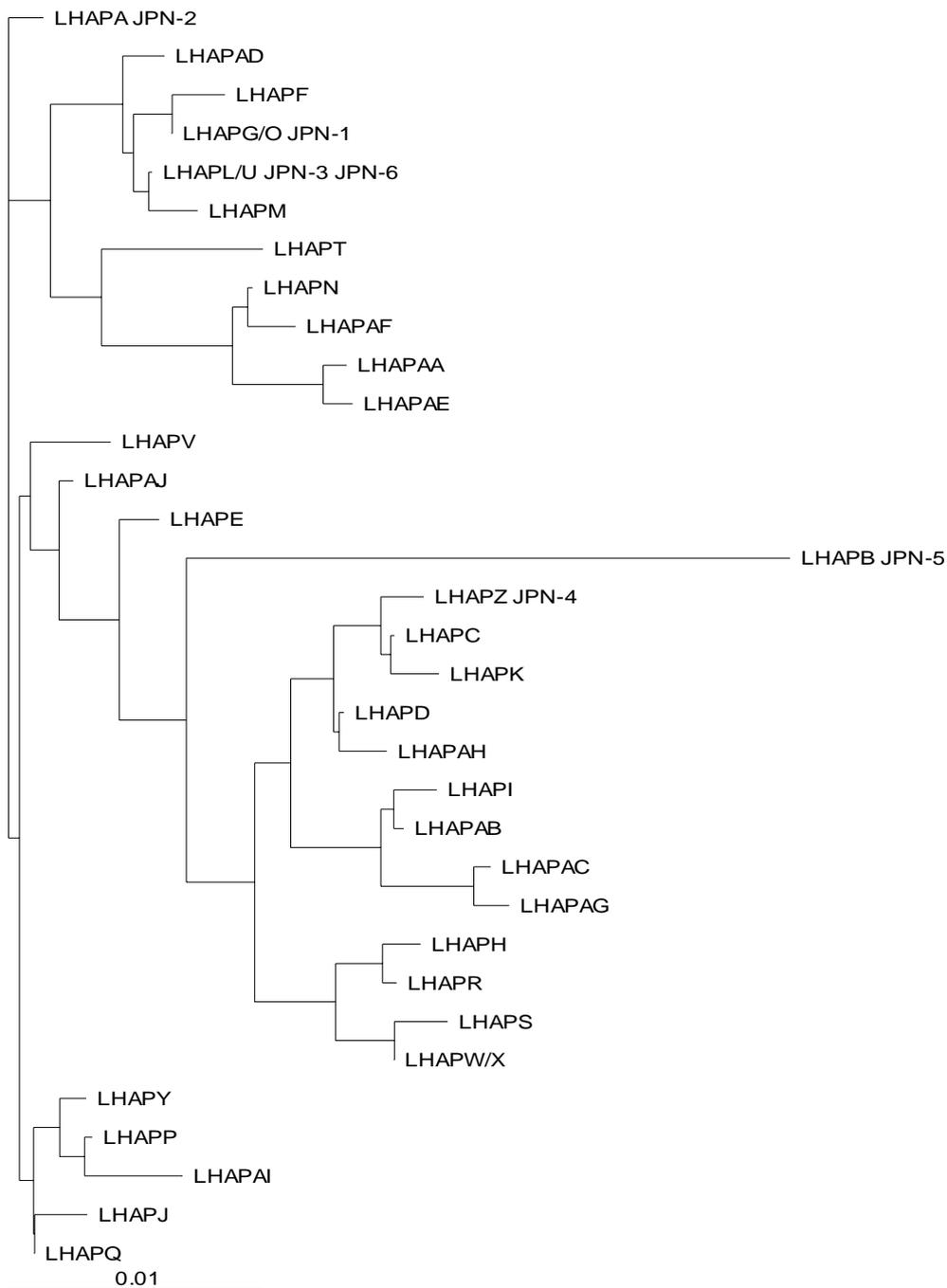


Fig. 2. Neighbour joining tree of the gray whales' mtDNA haplotypes.

LHAPs were extracted from Leduc *et al.* (2002). JPN-1 to JPN-5 was the Japanese gray whales 1 to 5 in Table 1.

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Table 1. Samples analyzed in this study.

	Eastern / Western	Ocean	Area	Year	Month	Sex	Body length
Leduc-E (N=120)*	Eastern	Bering/Pacific	Chukotka - California	1990th			
Leduc-W (N=45) *	Western	Okhotsk	Sakhalin	1990th			
JPN-1	Western	Pacific	Hokkaido	1995	Apr.	F	9.5
JPN-2	Western	Sea of Japan	Hokkaido	1996	May	---	---
JPN-3	Western	Pacific	Chiba	2005	May	F	7.8
JPN-4	Western	Pacific	Miyagi	2005	Jul.	F	12.8
JPN-5	Western	Pacific	Iwate	2007	Jan.	F	9.2
JPN-6	Western	Pacific	Hokkaido	2007	Aug.	F	12.3
RUS-23	Eastern	Bering	Chukotka	2008	Jul.	M	8.2
RUS-26	Eastern	Bering	Chukotka	2008	Aug.	M	8.1
RUS-28	Eastern	Bering	Chukotka	2008	Aug.	M	8.9
RUS-37	Eastern	Bering	Chukotka	2008	Aug.	F	12.0
RUS-39	Eastern	Bering	Chukotka	2008	Sep.	M	11.1
RUS-40	Eastern	Bering	Chukotka	2008	Sep.	F	12.3
RUS-47	Eastern	Bering	Chukotka	2008	Sep.	F	13.4

* Data from Leduc *et al.* (2002): Leduc-E corresponds to their eastern population sample, while Leduc-W to their western population one.

Table 2. Haplotype distributions within the samples used in this study.

	mtDNA haplotype																										Total								
	A	B	C	D	E	F	G/O	H	I	J	K	L/U	M	N	P	Q	R	S	T	V	W/X	Y	Z	AA	AB	AC		AD	AE	AF	AG	AH	AI	AJ	
Leduc-E	13	11	12	10	2	-	8	1	-	-	4	6	6	4	3	1	3	1	8	2	2	5	2	2	2	2	2	1	1	3	1	1	1	120	
Leduc-W	20	15	2	2	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	45
Japan	1	1	-	-	-	-	1	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	6	
Russia	-	-	2	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	1	-	1	-	-	-	7	

*Haplotype designation was according to Leduc *et al.* (2002).