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ABSTRACT

The number and distribution of North Pacific right whale populations is uncertain. Previous studies based on catch, stranding and sighting records suggested that two populations occur on either side of the North Pacific. In this study this hypothesis was tested by analyzing new and published mitochondrial DNA (mtDNA) control region sequences (399bp) of right whale individuals from the western (n=29) and eastern (n=23) North Pacific. A sub-sample from the western side (n=18) was examined with 13 microsatellite loci to investigate the level of nuclear DNA diversity. Striking mtDNA differences were found between western and eastern North Pacific right whales. The F_{ST} between western and eastern North Pacific right whales was high (0.0929) and statistically significant (P=0.0002). This result is consistent with the hypothesis that separate populations inhabit the eastern and western North Pacific. Levels of nucleotide and haplotype diversities were high, 0.0174/0.8916 and 0.0165/0.8538 in the western and eastern populations, respectively. For the microsatellite data, the average expected heterozygosity in the western population was estimated at 0.595. The observed multimodal mtDNA mismatch distribution rejected a model of historical sudden expansion in both populations. Furthermore, Bayesian skyline plots (BSP) generated from the mtDNA data suggested a similar historical trend of female effective population size (Ne_f) for the two populations, with a stable Ne_f over time followed by a recent sharp decline. The timing of the decline ranged between 25,000 and 60,000 years ago (considering different populations and two assumptions of mutation rates), which coincide with the last glaciation period in the Pleistocene. Rapid climate changes during this period could have affected the habitat and prey resources of the North Pacific right whales, resulting in the sharp decline in their abundance. No signal of recent recovery was observed in the BSP analysis; however, this could be due to a lack of resolution for contemporary population size as shown in other studies.

KEYWORDS: NORTHERN HEMISPHERE, NORTH PACIFIC RIGHT WHALE; GENETICS; CONSERVATION

INTRODUCTION

North Pacific right whales (*Eubalaena japonica*) occur during the summer in waters from the Okhotsk Sea, off southern eastern Kamchatka Peninsula, the Southern Bering Sea, and the northern Gulf of Alaska, south to the Sea of Japan, the Pacific coast of northern Honshu, and the coast of central California. Their distribution in winter includes the waters off Southern China, Taiwan, the Ogasawara Islands, and Baja California, Mexico (Rice, 1998). The species was heavily exploited by commercial whaling in the past (see details in Brownell *et al*., 2001; Scarff, 2001; Ivashenko & Clapham, 2012; Smith *et al*., 2012), and as a consequence the current populations of North Pacific right whales are considered severely depleted.

Smith *et al*. (2012) plotted on color maps the spatial distribution of America whaling and the targeted whale populations, based mainly on the original data in the studies of Maury (1852) and Townsend (1935). Figure 1 (reproduced from Smith *et al*., 2012) shows the daily location of whaling vessels with observations of right whales. In the North Pacific right whales were concentrated on either side of this ocean basin. There were also seasonal changes in distribution with whales occurring on both sides of the North Pacific in the periods March-May and June-August (Smith *et al*., 2012).

The interpretation of population trends as well as the development of sound conservation measures for North Pacific right whales requires information on the number and distribution of populations. However, to date the number of populations in the North Pacific right whale is uncertain. No study on population genetic structure at the ocean basin level is available, and the information on population structure from other non-genetic sources is very limited for this depleted species.

Omura (1958) examined the pattern of migration of right whales in the North Pacific based on sighting data collected in the period 1941-1957. He concluded that whales appear to the north-east of Honshu and south of Hokkaido in April staying there in May and then proceeding further north. In June whales arrive in the

Bering Sea and stay there for the whole summer. Omura (1958) noted that 'it is well known, however, that another stock of right whales than the western stock occurs in the eastern coast of the North Pacific', and that 'whales around the Aleutian Islands are without doubt belong to the stock in the so-called 'Kodiak Ground'. Gilmore (1956) had proposed previously a separate population near the coast of California. Similar conclusions on migration pattern were reached by Omura *et al*. (1969) who expanded the data used by Omura (1958) to include sighting records by Japanese catchers in the years 1958-1968.

More recently the patterns of catch and sighting distribution have been examined thoroughly, and the patterns have been interpreted in the context of population identity (Scarff, 1986; 1991; Brownell *et al*., 2001; Clapham *et al*., 2004). Scarff (1986; 1991) examined the 19th century whale charts of Maury (1852) *et seq*. and concluded that the population was not significantly denser close to shore, and that the data indicated no near-shore migration along the west coast of North America. The historical data from the 19th century suggested incidence of sighting in offshore waters, with no clear discontinuity through the North Pacific. A different conclusion was reached when more recent data were reviewed. Brownell *et al*. (2001) examined all available 20th century records of right whales in the North Pacific including sightings, stranding and catches. The data showed higher density on both sides of the North Pacific, and based on this observation, they supported the hypothesis that at least two populations of right whales exist, with one in the western North Pacific and another in the eastern North Pacific. Clapham *et al*. (2004) provided monthly plots of right whale sighting and catches from both the 19th and 20th centuries. They concluded that the pattern of north-south migratory movement reflected in the data supported the hypothesis of two largely discrete populations in the eastern and western North Pacific.

The right whale in the eastern and western North Pacific appear to have distinct catch and recovery histories, and this fact supports the idea that at least two populations exist in the feeding grounds on both sides of the North Pacific (Brownell *et al*., 2001). In fact, the population in the eastern North Pacific (southeastern Bering Sea shelf and south of Kodiak Island) was estimated to be approximately 30 whales (Wade *et al*., 2011; LeDuc *et al*., 2012). For the western North Pacific, Miyashita and Kato (1998) presented a preliminary estimate of 922 whales based on sighting survey conducted in the Okhotsk Sea in 1989, 1990 and 1992. Hakamada and Matsuoka (2016) estimated the abundance at 1,147 whales based on sighting data collected from May to June in 2011 and 2012, and at 416 whales based on surveys conducted from July to August in 2008 in a part of the western North Pacific, off southeastern Kamchatka Peninsula. However, these estimates have not been reviewed by the IWC Scientific Committee (IWC SC).

Based on the pattern of catches around Japan, Omura (1986) further suggested the occurrence of two populations on both sides of the Japanese Archipelago, one wintering in the Sea of Japan/East China Sea and summering in the Okhotsk Sea, and the other wintering in the Ryukyu Islands and summering off the northern Kuril Islands and in the western Bering Sea.

LeDuc *et al*. (2012) conducted the only genetic study of North Pacific right whales to date, but the study was based almost entirely on eastern North Pacific samples. The authors examined a total of 49 biopsy samples including 47 from the southeastern Bering Sea, one from the Gulf of Alaska and one from eastern Kamchatka Peninsula, which involved 23 individual whales (22[1] from the eastern NP and one from the western North Pacific). The study focused on the potential parentage and genetic diversity in the samples from the eastern North Pacific. The authors noted that one factor largely unexamined to date was the relationship of the eastern North Pacific right whales to those in the western North Pacific, and that gene flow from the west could potentially mitigate some of the problems faced by the whales in the eastern side (LeDuc *et al*., 2012).

The main objective of this study was to test the hypothesis of two populations by analyzing genetic samples of right whales from the eastern and western North Pacific. The hypothesis of Omura (1986) of two different populations on both sides of the Japanese Archipelago could not be tested in this study as only a single sample was available from the Sea of Japan. A secondary objective was to investigate the historical population trend in right whales from the western and eastern sides of the North Pacific, and the possibility

[1] This number differs from that reported in LeDuc *et al*. (2012) (n=23 whales in the ENP) as re-assessment of the microsatellite data revealed that two samples previously considered to have been collected from different individuals had instead been collected from the same animal (Lang, pers. comm).

of any historical decline. The relevance of this is that sharp decline could lead to a loss of genetic variability and elevated levels of inbreeding, increasing the risk of extinction and compromising adaptive evolutionary potential.

MATERIALS AND METHODS

Samples

The new genetic samples examined in the present study were 34 skin and baleen plates of right whales collected from the western (n=31) and eastern (n=3) sides of the North Pacific, and they were from different sources (Table 1).

DNA extraction

The International Whaling Commission Scientific Committee (IWC SC) guidelines for DNA quality (IWC, 2009) were followed as much as possible (Kanda *et al*., 2014). Genomic DNA was extracted from approximately 0.05g of the outer epidermal layer of the skin tissue using standard protocols (Sambrook *et al*., 1989) or using Gentra Puregene kits (QIAGEN). In the case of baleen plates, DNA was extracted using the Bio 101 'Genclean Kit for Ancient DNA'. Extracted DNA was stored in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0).

Laboratory procedures

mtDNA

The first 470 nucleotides at the 5' end of the mtDNA control region were amplified by polymerase chain reaction. The oligo-nucleotides employed in the PCR amplification were MT4 (Arnason et al., 1993) and Dlp 5R (5'-CCA TCG AGA TGT CTT ATT TAA GGG GAA C-3'). Reactions were carried out in 25µL volumes containing 10-100ng of DNA, 2.5pmole of each primer, 0.5 units of Ex Taq DNA polymerase (Takara), 2mM of each dNTP, and 10x reaction buffer. After an initial denaturation step at 95°C for 5 minutes, a PCR amplification cycle of 30 seconds at 94°C, followed by 30 seconds at 50°C and 30 seconds at 72°C are repeated 30 times. The amplification was completed with a final extension step of 10 minutes at 72°C. Subsequent cycle sequencing reactions were performed with 100ng of products generated in the above PCR amplifications using the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems). The oligo-nucleotides used to prime the cycle sequencing reaction were the same as employed in the initial PCR amplification listed above. A total of 25 cycles for 10 seconds at 96°C, 20 seconds at 56°C and four minutes at 60°C were performed. The nucleotide sequence of each cycle sequencing reaction was determined using Applied Biosystems 3500 Genetic Analyzer (Life Technology) under standard conditions. Both strand samples were sequenced in their entirety for all samples.

Microsatellite DNA

Genetic variation was examined at 13 microsatellite loci: EV1, EV14, EV21, EV37, EV94 (Valsecchi and Amos, 1996), GT23, GT211, GT310 (Bérubé *et al*., 2000), GATA28 (Palsbøll *et al*., 1997), DlrFCB17 (Buchanan *et al*., 1996), TR3G2, TR2G5 and TR3F2 (Frasier *et al*., 2006). The SRY locus located on the Y chromosome was also used for sex determination following the method of Abe *et al*. (2001) with a slight modification. With the combination of loci of SRY and GT23, males show amplified products of both SRY and GT23 loci, while females show only GT23. Although primer sequences followed those of the original authors, an annealing temperature of each of the loci was optimized for the species. Multiplex PCR amplifications were performed in 15µl reaction mixtures containing 10-100ng of DNA, 5 pmole of each primer, 0.625 units of Ex Taq DNA polymerase (Takara), and 2mM of each dNTP, and 10x reaction buffer containing 20mM $MgCl_2$ (Takara). All PCR products were electrophoresed on an Applied Biosystems 3500 Genetic Analyzer. Allele sizes were determined using a 600 LIZ size standard and GeneMapper v. 5.0 (ABI).

Analytical procedures

Some tissue and DNA samples were lost due to the 2011 tsunami in Japan and therefore those samples were not available for the microsatellite analyses. While all 34 samples were available for the mtDNA analysis, only 19 samples were available for the microsatellite analysis.

mtDNA

After removing duplicates and one sample from each mother and calf pair (see below), the sample sizes for the analyses were 28 and three for the western and eastern North Pacific, respectively. LeDuc *et al*. (2012)'s samples involved two cases of mother and calf pairs (see below), then their sample sizes for the analyses were 20 and one for the eastern and western North Pacific, respectively. After pooling the samples with those of LeDuc *et al*. (2012), the total sample size for the western and eastern North Pacific became 29 and 23, respectively (Figure 2).

MtDNA sequences from this study and from LeDuc *et al*. (2012) were aligned to produce a single data set. Sequences were aligned by eye using Sequence Navigator (Applied Biosystem, Inc). Variable sites and unique sequences (haplotypes) were identified using the program MacClade (Maddison and Maddison, 1989).

The degree of mtDNA intrapopulation diversity was estimated using the number of haplotypes, nucleotide and haplotype diversities (Nei, 1987) using the computer program ARLEQUIN ver. 3.5 (Excoffier and Lischer, 2010). The degree of spatial mtDNA divergence between western and eastern North Pacific whales was estimated via the conventional FST using the program ARLEQUIN ver. 3.5 (Excoffier and Lischer, 2010). Statistical significance was obtained by 10,000 Monte Carlo simulation. Heterogeneity test was conducted by the randomized chi-square (Roff and Bentzen, 1989) using the program R. The level of statistical significance was estimated from 10,000 Monte Carlo simulations as the proportion of simulations in which a similar or more extreme value of chi-square was observed.

A statistical parsimony haplotype network was constructed with a 95% connection limit using the computer program TCS v1.21 (Clement et al., 2000) to infer phylogenetic relationships among mtDNA haplotypes.

Mismatch distribution analysis (Rogers and Harpending, 1992; Excoffier, 2004) was conducted using the program ARLEQUIN to infer historical sudden population expansion. The observed distribution of pairwise nucleotide differences among individuals was compared with the expected distributions under the Sudden Expansion model (Rogers and Harpending, 1992; Rogers, 1995), and evaluated with goodness-of-fit tests (sum of squared deviations: Schneider and Excoffier, 1999).

In addition, a Bayesian Skyline Plots (BSP), which uses the coalescent theory to infer changes in effective population (*Ne*) through time, was constructed. The BSP was constructed using the program BEAST v. 2.4.6, with 10 million MCMC generations that yielded effective sample sizes of at least 200. In the Tracer v1.5 Software, the marginal densities of temporal splits were analyzed and the Bayesian Skyline reconstruction option was selected for the trees log file. A mutation rate and generation time were required to convert the parameter estimates obtained from the BSP analyses into absolute numbers and time. Mutation rates of 7×10^{-8} (Harlin *et al*., 2003) and 1×10^{-8} substitutions/site/year (Hoelzel, 1993), and a generation time of 29.8 years (Taylor *et al*., 2007), were used.

Microsatellite DNA

After excluding one sample from a mother and calf pair (see below), a total of 18 samples from the western North Pacific were available for the microsatellite analyses.

The computer program MICRO-CHECKER (van Oosterhout et al., 2004) was used to check for null alleles and reading/typing errors. The number of alleles per locus, inbreeding coefficient (FIS) and expected heterozygosity (HE) per locus was calculated using FSTAT 2.9.3 (Goudet, 1995). Statistical tests for the deviations from expected Hardy-Weinberg genotypic proportions were conducted using GENEPOP 4.0 (Rousset, 2008), and this was done by each locus as well for all loci combined. A FDR correction (Benjamini and Yekutieli, 2001) was used to adjust the significance level for all multiple comparisons in the present study.

RESULTS

Duplicates

No duplicates were found among the biopsy samples for which the microsatellite data were available. Microsatellite data were not available for biopsy samples B04NPRI01-B04NPRI02 in Table 1. Both samples were obtained from the same school, and exhibited the same mtDNA haplotype. Therefore, this likely represented a duplicate and consequently only one of these samples was considered in the subsequent mtDNA statistical analyses.

Mother and calf pairs

Western North Pacific

Microsatellite data were not available for biopsy samples B05NPRI01-B05NPRI02 in Table 1. Both samples were obtained from the same school and exhibited the same mtDNA haplotype. The body sizes of the animals were 16.2m and 8.2m (Table 1). It was concluded that the two samples were likely from a mother and calf pair. Only one of the individuals was considered in the subsequent mtDNA statistical analyses.

There was another case of a mother and calf pair determined by the microsatellite analysis (biopsy samples 11NPSRI06 and 11NPSRI07 in Table 1). Both samples exhibited the same mtDNA haplotype. Only one of the individuals was considered in the subsequent mtDNA statistical analyses.

Based on body length data, there were two possible cases of calf animals in the bycatch sample (Table 1).

Eastern North Pacific

The samples from LeDuc *et al*. (2012) involved two cases of mother and calf pairs. Only one of each pair of samples was used in the subsequent mtDNA statistical analysis.

Sex ratio

Among the 29 individual whales from the western North Pacific (28 from this study and one from LeDuc *et al*., 2012), sex information was available for 21 whales, and the male sex ratio was 0.38 (Table 1).

mtDNA diversity

The final data set included the first 399 nucleotides of the mtDNA control region. A total of 16 haplotypes was found in the full sample of 52 individual whales. This number was derived from 25 transitions and one insertion/deletion (Table 2). The sequences of the nine new haplotypes have been deposited in GenBank under accession numbers XXX-XXX, respectively (Table 2).

Table 3 shows the number of individual whales, number of haplotypes, number of singletons, and nucleotide and haplotype diversities for right whales in the western and eastern North Pacific. Both nucleotide and haplotype diversity indices indicated a relatively high diversity. Each index had fairly similar values for the western and eastern North Pacific right whales. However, for a similar number of samples, the numbers of haplotypes and singletons were higher in the western North Pacific whales.

Geographical distribution of mtDNA haplotypes

Table 2 shows the geographical distribution of the 16 haplotypes. Only three haplotypes, including the most frequent haplotype '2', were shared between western and eastern North Pacific right whales. Nine haplotypes involving 24 individuals were specific to the western North Pacific while four haplotypes involving 13 individuals were specific to the eastern North Pacific.

Five North Pacific right whale samples (NP baleen 1, 2, 3, 4 and 5 in Table 1) were obtained during the 1950's and 1960's under special scientific permit whaling. NP baleen 1 and 2 were collected in the western North Pacific, and these exhibited haplotypes 14 and 9, respectively. Haplotypes 14 and 9 each occurred in three individuals of the western North Pacific. NP baleen 3, 4 and 5 were collected in the eastern North Pacific, and these exhibited haplotypes 2, 7 and 6, respectively. Haplotype 2 was a common haplotype in both western and eastern North Pacific; haplotype 7 was a singleton in the eastern North Pacific and haplotype 6 was a common haplotype in the eastern North Pacific but also occurred in lower frequency in the western North Pacific.

mtDNA heterogeneity test
The F_{ST} between western and eastern North Pacific right whales was high (0.0929) and statistical significant (P=0.0002), and the heterogeneity test based on the randomized chi-square test resulted also in significant statistical differences between whales in both sides of the North Pacific (χ^2 = 37.72; P < 0.0001).

Evolutionary relationship among mtDNA haplotypes
Figure 3 shows the parsimony network of 16 mtDNA haplotypes in the North Pacific right whales. The network consisted mainly of three haplotype groups, which were connected by several mutation steps. Although some haplotype genealogies in the network were specific to each population, a distinct geographic concordance of the network was not observed.

Historical demography
The mismatch distribution was multimodal in both eastern (Figure 4a) and western (Figure 4b) North Pacific populations. The results of goodness-of-fit tests for the observed mismatch distributions significantly deviated from those of the simulated distribution under the Sudden Expansion model in both cases (P < 0.05), suggesting that the North Pacific right whale did not undergo the population expansion assumed in the model.

The BSP shows a stable Ne_f over time followed by a recent sharp decline, and this trend was similar in both western and eastern North Pacific populations (Figure 5). While the same pattern was seen in the results for both mutation rates, the absolute values for the timing to start demographic decline and the Ne_f of the two populations varied according the mutation rate used (Table 4), which were estimated to be in the range of 25,000-40,000 years ago with 8,300-12,000 female individuals in the western population and 25,000-60,000 years ago with 4,500-11,000 female individuals in the eastern population (Table 4).

Microsatellite DNA diversity
Table 5 shows the microsatellite DNA diversity indices in the western North Pacific right whale. The number of alleles per locus ranged from 2 to 7 (4.15 in average), and the H_E ranged from 0.255 to 0.786 (0.595 in average). The F_{IS} in each locus ranged from -0.199 to 0.294 with 0.023 on average. There was no significant deviation from the Hardy-Weinberg genotypic proportion (χ^2 = 30.67; *d.f.* = 26.00; P = 0.241).

DISCUSSION

The main results of the present study can be summarized as follows: a) mtDNA analyses support the hypothesis of two different populations on each side of the North Pacific; b) the observed mismatch distribution in both eastern and western North Pacific populations rejects a model of historical sudden population expansion; c) results of the Bayesian skyline plot suggest a demographic pattern of a historically stable Ne_f, followed by a more recent sharp decline in both populations; d) historical abundance is slightly higher and the timing to start demographic decline is more recent in the western population than in the eastern population; and e) the current mtDNA diversity is high. The main results are discussed below.

Population structure
The results of the mtDNA analysis showed striking genetic differences between western and eastern North Pacific right whales, which is consistent with the population specific genealogies observed in the haplotype network which appear to evolve independently in each population (see for example haplotypes '13'and '14'; '8', '9' and '11'; and '10' and '15' in Figure 3). These results are consistent with the pattern of 20th century catch and sighting data showing higher density on both sides of the North Pacific (Brownell *et al*., 2001). Therefore, the pattern of modern catch, stranding and sighting distribution and the genetic data support the hypothesis of two distinct populations occurring on both sides of the North Pacific. While the migration pattern of these two populations has been described by several authors (Gilmore, 1956; Omura, 1958; Omura *et al*., 1969; Clapham *et al*., 2004), the lack of genetic material prevented the investigation of possible population boundary or areas of geographical overlap between the two populations in the central part of the North Pacific. It should be noted that the comparative genetic analysis between western and eastern North Pacific right whales was based on a single marker, mtDNA, and that future comparisons should be based on nuclear DNA as well. As indicated previously only one genetic sample was available from a right whale from the Sea of Japan, and that whale exhibited haplotype '2', which was the most

common haplotype occurring in both western and eastern populations. As stated earlier, in the present study it was not possible to test the hypothesis of a different population of right whales in the Sea of Japan (Omura, 1986).

Genetic diversity

The nucleotide and haplotype diversities were high in comparison with those reported for other more abundant baleen whale species in the North Pacific. The haplotype network showed three haplotype groups which were connected by several mutation steps. However, those groups included whales from both eastern and western populations and therefore there was not a concordance between geography and network haplotype pattern. The pattern observed could be explained by incomplete lineage sorting of ancestral polymorphisms resulting from relatively recent divergence and/or large historical population sizes, which could explain also the high level of haplotype and nucleotide diversities in both populations (see Table 3) despite of historical decline and the 19th century high whaling pressure.

Regarding microsatellites diversity, the average heterozygosity in the western North Pacific population almost doubles the levels of diversity estimated for the critically endangered North Atlantic right whales (Waldick *et al*., 2002).

Historical demography

The BSP analyses inferred that the western and eastern North Pacific right whale populations underwent a drastic decline in N_{ef} which started from 25,000-40,000 and 25,000-60,000 years ago, respectively. This inference did not contradict the observed mismatch distribution rejecting a population expansion model, and puts the decline in the late Pleistocene, specifically during the last glaciation period which started 115,000 years ago and ended 11,500 years ago, with the Last Glacial Maximum occurring around 22,000 years ago.

It is known that the Quaternary ice ages impacted the genetic structure of many living organisms ranging from the Arctic to temperate regions (Hewitt, 2000). Foote *et al*. (2013) predicted the shift of core suitable habitat of the bowhead and North Atlantic right whales during the late Pleistocene into Holocene using the habitat model, which suggested that the core habitat of the Atlantic right whale was restricted southward of 50°N during the late Pleistocene. In the case of North Pacific right whales, all of their current feeding ground, especially in the Bering Sea and parts of the Gulf of Alaska, were unavailable during the late Pleistocene. This could have implied a low availability of prey resources. Considering that the North Pacific right whales feed on zooplanktons (Omura, 1986), e.g., copepods *Calanus marshallae, Metridia* spp., and *Neocalanus* spp. and the euphausiid *Euphausia pacifica*, the loss of a part of their feeding ground due to glaciation could cause the demographic reduction of this species. Therefore, the fluctuating climate change in the Pleistocene is one possible explanation for the sharp demographic decline observed in this study, as hypothesized for populations of the killer whale (Moura *et al*., 2014).

The historical reduction in Ne_f was observed across BSPs generated from multiple analyses that differed in assumptions regarding mutations rates. It is important to note that no signal of post-decline recovery was observed by the BSP analyses. However, this could be due to a lack of resolution for contemporary population size as showed in other studies (Hoffman, *et al*., 2011). In fact, recent sighting information for the western North Pacific population showed that the number of whales sighted by 100n. miles increased from 1994 to 2016 (Matsuoka *et al*., 2017).

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Table 1. Details of the new samples (e.g. other that those used in LeDuc *et al*., 2012) of North Pacific right whale examined in this study. The abbreviation is as follows: M, male; F, female; Uk, unidentified; No Msat, there is no data of microsatellites; Dup, duplicate; MC, mother and calf pair. Total length of whales biopsied was estimated from the vessels at the time the samples were collected.

Sample ID	Source	Year	Month	Day	Latitude	Longitude	Sex	Body length (m)	School size	Remarks
03BC034	Bycatch	2003	4	1	33 28.3 N	135 50.3 E	M	13.6		
11BC025	Bycatch	2011	2	27	32 46.6 N	131 56.4 E	Uk	7.0		No Msat
16BC119	Bycatch	2016	10	16	42 07.2 N	140 37.2 E	M	9.5		
BJ-M-1067	Stranding	2007	1	28	36 07.0 N	136 03.0 E	Uk			No Msat
BJ-M-1408	Stranding	2009	3	26	35 46.0 N	140 49.0 E	Uk			No Msat
BJ-M-456	Stranding	2003	4	1	36 40.0 N	140 41.0 E	M	13.0		No Msat
BJ-M-738	Stranding	2005	2	21	34 45.0 N	139 21.0 E	F	10.0		No Msat
B03NPRI01	Biopsy	2003	7	31	42 12.6 N	167 25.3 E	Uk	15.0	1	No Msat
B04NPRI01	Biopsy	2004	8	4	48 53.8 N	162 14.2 E	Uk	12.7, 11.3	2	No Msat/Dup
B04NPRI02	Biopsy	2004	8	4	48 53.8 N	162 14.2 E	Uk	12.7, 11.3	2	No Msat/Dup
B05NPRI01	Biopsy	2005	7	23	43 17.3 N	155 05.3 E	Uk	16.2	2	No Msat/MC
B05NPRI02	Biopsy	2005	7	23	43 17.3 N	155 05.3 E	Uk	8.2	2	No Msat/MC
B12NPRI01	Biopsy	2012	6	6	40 06.5 N	143 17.8 E	M	13.8	1	
B15NPRI001	Biopsy	2015	4	29	47 20.5 N	159 58.3 E	F	13.0	1	
B15NPRI002	Biopsy	2015	5	9	46 12.0 N	164 24.1 E	F	13.1	1	
11ENPSRI01	Biopsy	2011	5	19	42 03.6 N	151 21.5 E	F	13.3	1	
11ENPSRI02	Biopsy	2011	5	19	42 10.1 N	151 23.7 E	M	13.5	2	
11NPSRI01	Biopsy	2011	5	21	48 03.0 N	163 27.7 E	F	10.7	1	
11NPSRI02	Biopsy	2011	5	21	46 51.8 N	163 08.1 E	F	12.3	1	
11NPSRI03	Biopsy	2011	5	27	46 37.7 N	160 34.1 E	F	13.4	2	
11NPSRI04	Biopsy	2011	5	27	46 40.8 N	160 32.7 E	M	13.0	2	
11NPSRI05	Biopsy	2011	5	27	46 56.9 N	160 25.6 E	F	14.1	1	
11NPSRI06	Biopsy	2011	5	28	47 34.6 N	160 08.9 E	F	14.8	1	M
11NPSRI07	Biopsy	2011	5	28	47 35.1 N	160 10.0 E	F	11.0	1	C
11NPSRI08	Biopsy	2011	5	30	46 02.8 N	159 04.6 E	F	14.2	3	
11NPSRI09	Biopsy	2011	5	30	46 02.8 N	159 04.6 E	M	10.8	3	
11NPSRI10	Biopsy	2011	5	30	46 02.8 N	159 04.6 E	F	13.9	3	
11NPSRI11	Biopsy	2011	5	30	45 31.5 N	158 51.0 E	M	13.6	2	
11NPSRI12	Biopsy	2011	5	30	45 17.9 N	158 45.2 E	F	13.8	2	
NPBaleen1	Historical special permitt whaling	1968	7	25	48 14.0 N	146 39.0 E	F	12.6		No Msat
NPBaleen2	Historical special permitt whaling	1956	5	23	38 32.0 N	143 40.0 E	F	11.7		No Msat
NPBaleen3	Historical special permitt whaling	1962	7	30	53 42.0 N	171 17.0 W	F	14.1		No Msat
NPBaleen4	Historical special permitt whaling	1961	8	22	55 53.0 N	153 06.0 W	M	15.1		No Msat
NPBaleen5	Historical special permitt whaling	1961	8	22	55 54.0 N	153 07.0 W	M	17.1		No Msat

Table 2. Variable sites defining 16 mtDNA haplotypes in North Pacific right whales. Nucleotide position of the polymorphic sites starts from the 5' end of the mtDNA control region. Haplotypes '2' through '16' are listed with reference to haplotype '1'. A dot indicates an identical nucleotide at the position relative to haplotype '1'.

Haplotype ID	Nucleotide position of the polymorphic sites			Frequency			Accession No.
	11	1111122222	223333	Western North Pacific	Eastern North Pacific	Total	
	266789900	1244802488	891225				
	6448965604	1634868645	623124				
1	AGCTTCGTTA	GTCTGTCAAC	TTAAGC	0	3	3	JX441356 (LeDuc *et al.* , 2012)
2	.........G	.......G.T	......	4	5	9	JX441357 (LeDuc *et al* ., 2012)
3	-...C.AC.G	A...ACT...	..G...	0	6	6	JX441358 (LeDuc *et al.* , 2012)
4	.....T.C.G	AC..ACT...	CCG...	1	1	2	JX441359 (LeDuc *et al* ., 2012)
5	....C...C.	....ACT...	CCG...	0	3	3	JX441360 (LeDuc *et al* ., 2012)
6	....C...C.	....A.T...	CCG...	1	4	5	JX441361 (LeDuc *et al.* , 2012)
7	....C..C.G	....ACT...	CCG...	0	1	1	Present study
8	.A..C...CG	....A.T...	C.G..T	8	0	8	JX441362 (LeDuc *et al* ., 2012)
9	....C...CG	....A.T...	C.G..T	3	0	3	Present study
10	....C..C.G	A.T.ACT.G.	......	2	0	2	Present study
11	....C...C.	....A.T...	C.G..T	1	0	1	Present study
12	.......C..	A...ACT...	CCG...	1	0	1	Present study
13	.........G	....A.T...	...GA.	1	0	1	Present study
14	.........G	....A.T...	...G..	3	0	3	Present study
15	...C...C..	...CACT.G.	......	3	0	3	Present study
16	..T.CT..CG	....A.T...	CC....	1	0	1	Present study
Total				29	23	52	

Table 3. Indices of mtDNA diversity in North Pacific right whales.

Population	n	Number of haplotypes	Number of singletons	Nucleotide diversity (SE)	Haplotype diversity (SE)
Western North Pacific	29	12	4	0.0174 (0.0014)	0.8916 (0.0069)
Eastern North Pacific	23	7	1	0.0165 (0.0009)	0.8538 (0.0075)

Table 4. Summary of historical demographic inference from the Bayesian skyline plot analysis.

Demographic inference	Population	Mutation rate (substitutions/year/site)	
		7×10^{-8}	1×10^{-8}
Timing to start demographic decline (years ago)	Western North Pacific	25,000–35,000	30,000–40,000
	Eastern North Pacific	25,000–30,000	40,000–60,000
Approximate *Nef* (individuals)	Western North Pacific	8,300	12,000
	Eastern North Pacific	4,500	11,000

Table 5. Indices of microsatellite DNA diversity in western North Pacific right whales: n,number of samples; A, number of alleles; F_{IS}, inbreeding coefficient; H_E, expected heterozygosity; HW, *P*-value for the test of Hardy-Weinberg equilibrium

Microsatellite loci	n	A	F_{IS}	H_E	HW
EV1	18	5	0.124	0.761	0.816
GT310	18	3	0.294	0.629	0.018
GT23	18	5	-0.114	0.748	0.930
EV94	18	3	-0.090	0.255	1.000
EV14	18	4	0.108	0.498	0.155
GT211	18	3	0.016	0.508	0.144
EV37	18	5	0.010	0.786	0.169
GATA28	18	7	-0.035	0.752	0.122
EV21	18	2	-0.199	0.510	0.636
DlrFCB17	18	6	0.176	0.742	0.172
TR3G2	18	5	-0.138	0.732	0.517
TR2G5	18	3	0.185	0.477	0.613
TR3F2	18	3	-0.155	0.337	1.000
Overall	18	4.15	0.023	0.595	0.241

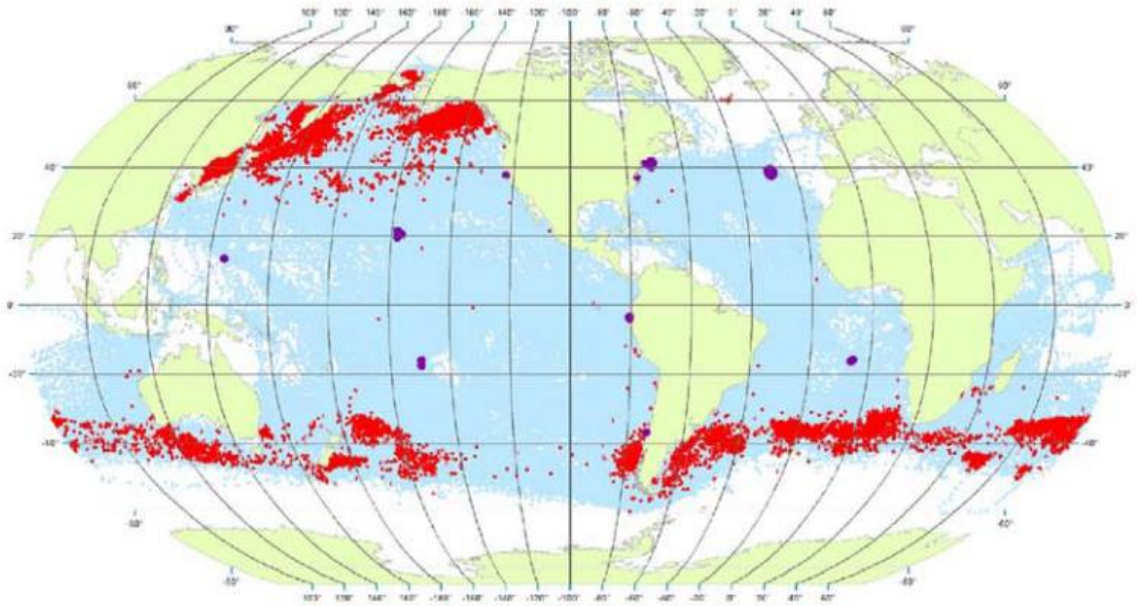

Figure 1. Daily locations of whaling vessels with observations of right whales based on American whaling logbooks for voyages departing between 1780 and 1920 (extracted from Smith *et al*., 2012).

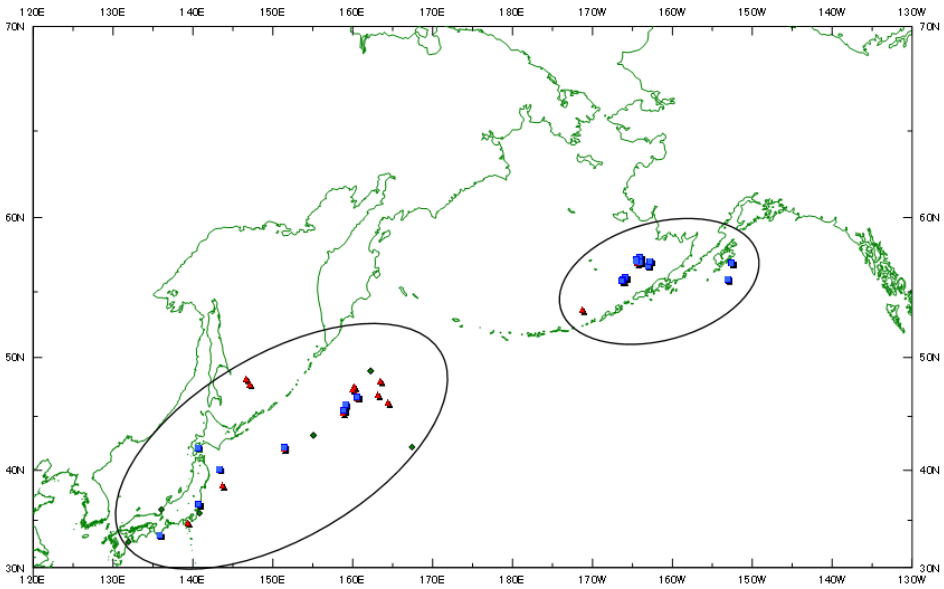

Figure 2. Geographical distribution of the North Pacific right whales examined in this study. Most of the samples from the eastern side are from LeDuc *et al*. (2012). Blue square: male; Red triangle: female; Green diamond: sex unknown. Solid circles identify the groups of right whales from the western and eastern sides of the North Pacific, used for the comparative genetic analysis. This grouping is based on previous information on distribution of catches and sightings (see Introduction).

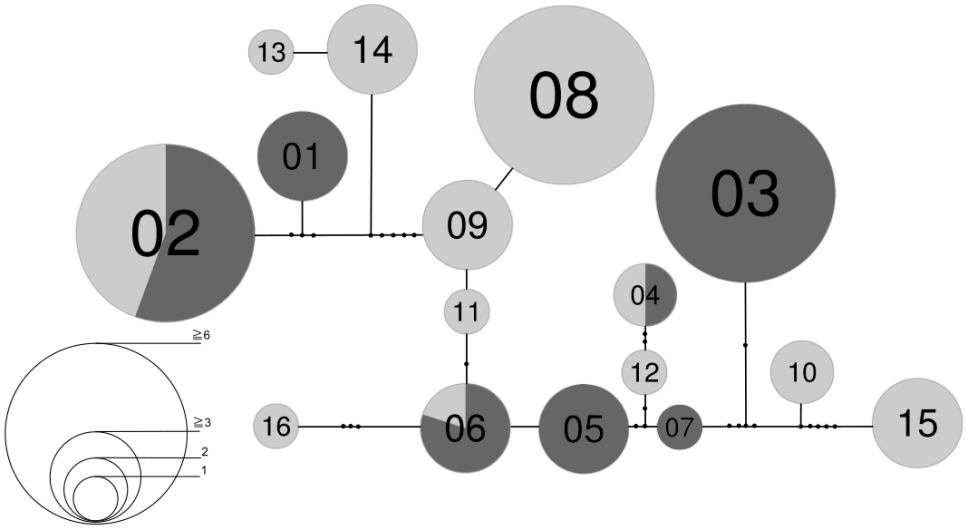

Figure 3. Statistical parsimony network based on 16 mtDNA haplotypes of North Pacific right whales. Each line and circle respectively indicates a single mutational step and haplotype. Circle size refers to haplotype abundance and populations are shaded: dark grey, eastern North Pacific; light gray, western North Pacific. Small filled circles indicate intermediate haplotypes not found in this study.

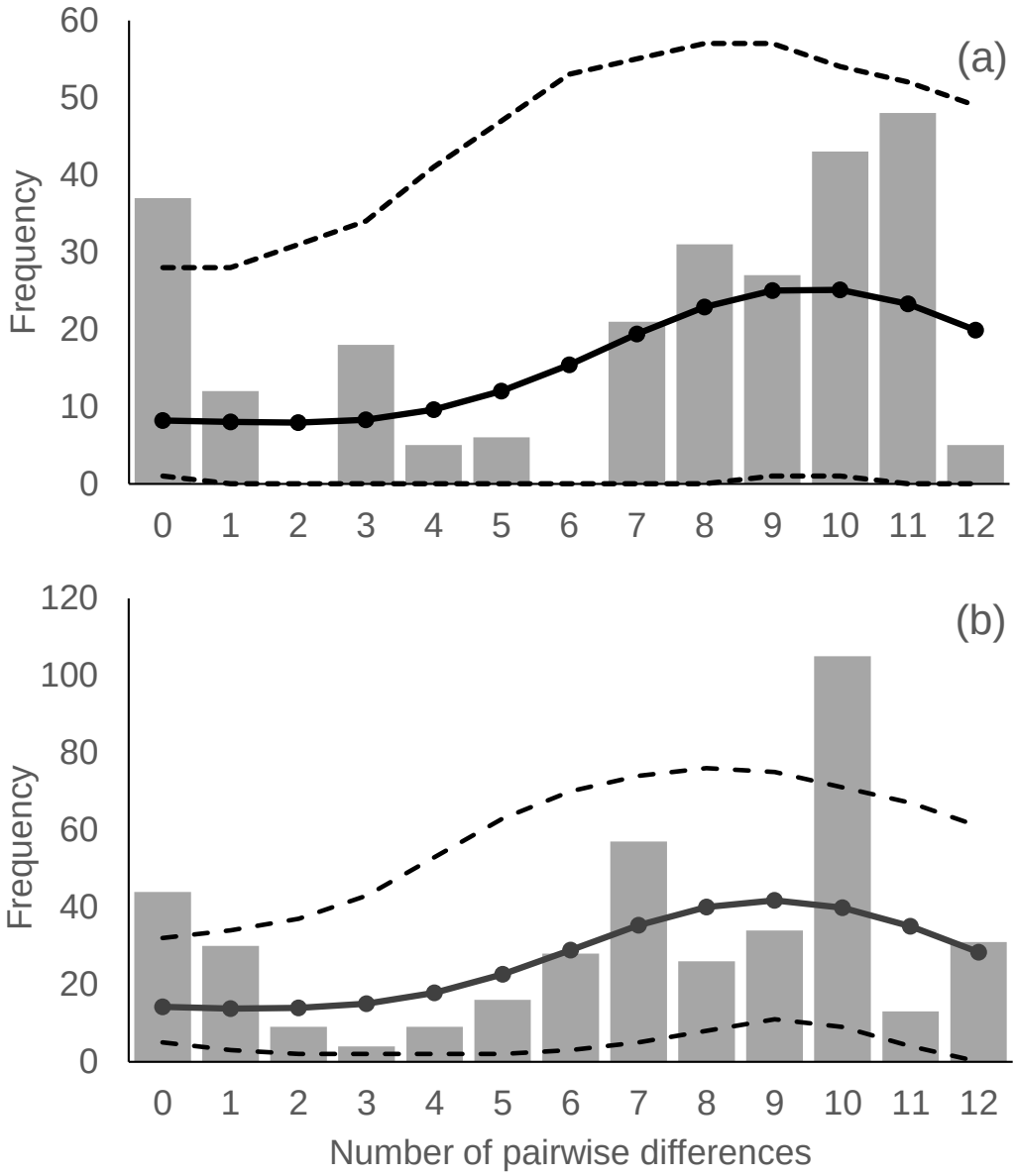

Figure 4. Mismatch distributions under the sudden expansion model: (a) Eastern North Pacific, (b) Western North Pacific. The bar and solid line show the observed and expected distributions, respectively, and dashed lines indicate upper and lower 95% confidence intervals around the expected distribution.

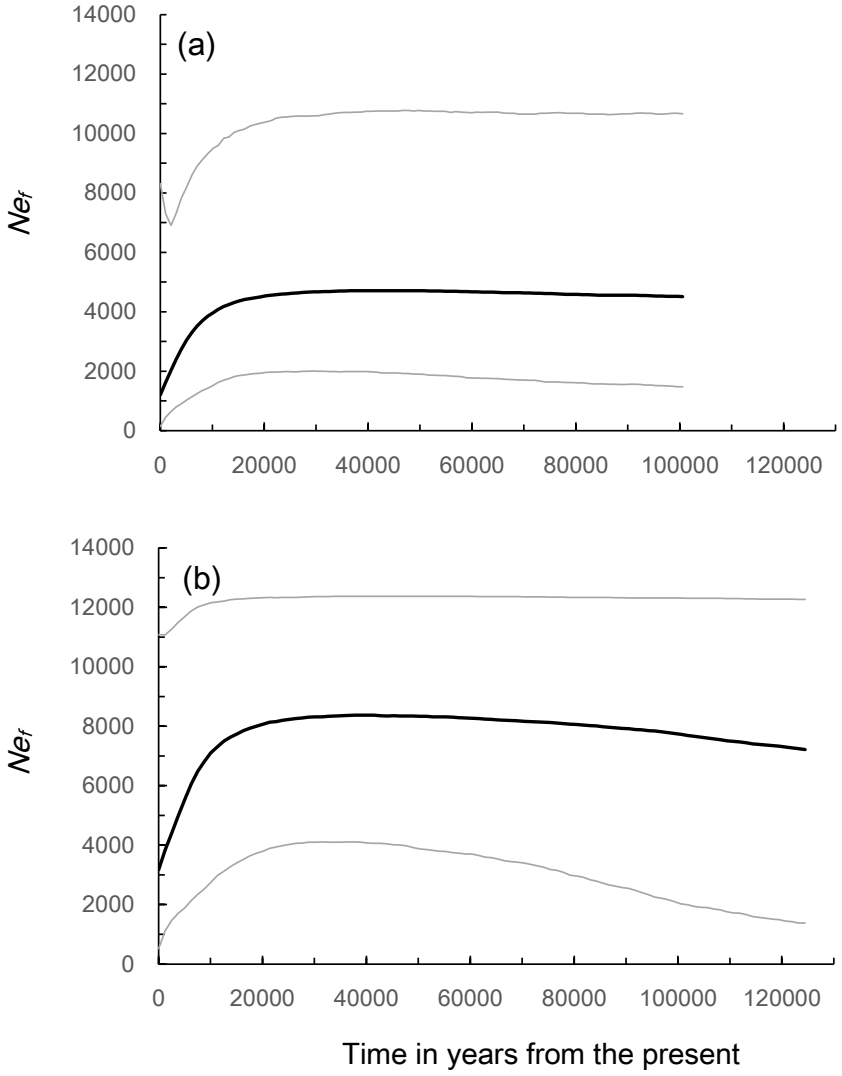

Figure 5. Results of Bayesian skyline plot analysis for North Pacific right whales using a mutation rate of 7 x 10^{-8} substitutions/site/year (Harlin *et al*. 2003) and a generation time of 29.8 years (Taylor *et al*. 2007): (a) Eastern North Pacific, (b) Western North Pacific. Black line indicates the median of female effective population size (Ne_f), and the gray lines show the upper and lower bound of 95% high posterior density.