Population genetic structure of North Pacific right whales

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North Pacific right whales (*Eubalaena japonica*) occur during the summer in waters from the Okhotsk Sea, Kuril Islands, off southern and southeastern Kamchatka Peninsula, the southeastern Bering Sea, the northern Gulf of Alaska, and the Pacific coast of northern Honshu (Figure 1). Their known current distribution in winter includes the waters off Southern China, Taiwan, the Ogasawara Islands, Hawaii, and Baja California, Mexico (Gendron et al., 1999; Herman et al., 1980; Kennedy et al., 2011; Rice, 1998). The species was heavily exploited by commercial whaling in the past and is now considered severely depleted, particularly the whales in the eastern North Pacific, which are designated on the IUCN Red List as "Critically Endangered" (Cooke & Clapham, 2018).

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

Most of what is known about population structure in North Pacific right whales has been based on the distribution of sighting records and whaling catch data. Josephson et al. (2008) reviewed historical catch data and concluded that right whales were likely not distributed continuously across the North Pacific but instead had a pronounced longitudinal bimodal distribution and were encountered infrequently in the central-northern North Pacific. Smith et al. (2012) further explored the historical catch data by plotting the spatial distribution of American whaling and the targeted whale populations, based mainly on the original data in the studies of Maury (1852) and Townsend (1935). Right whales were concentrated on either side of the North Pacific. There were also seasonal changes in distribution, with whales occurring on both sides of the North Pacific in the Northern hemisphere spring (March–May) and summer (June–August) (Smith et al., 2012).

In the western North Pacific, Omura (1958) examined the pattern of migration of right whales based on sighting data collected from 1941 to 1957. He concluded that whales appear to the northeast of Honshu and south of Hokkaido in April staying there in May and then proceeding farther north. In June, whales arrive in the Bering Sea and stay there for the summer. Omura (1958) noted that "it is well known, however, that another stock of right

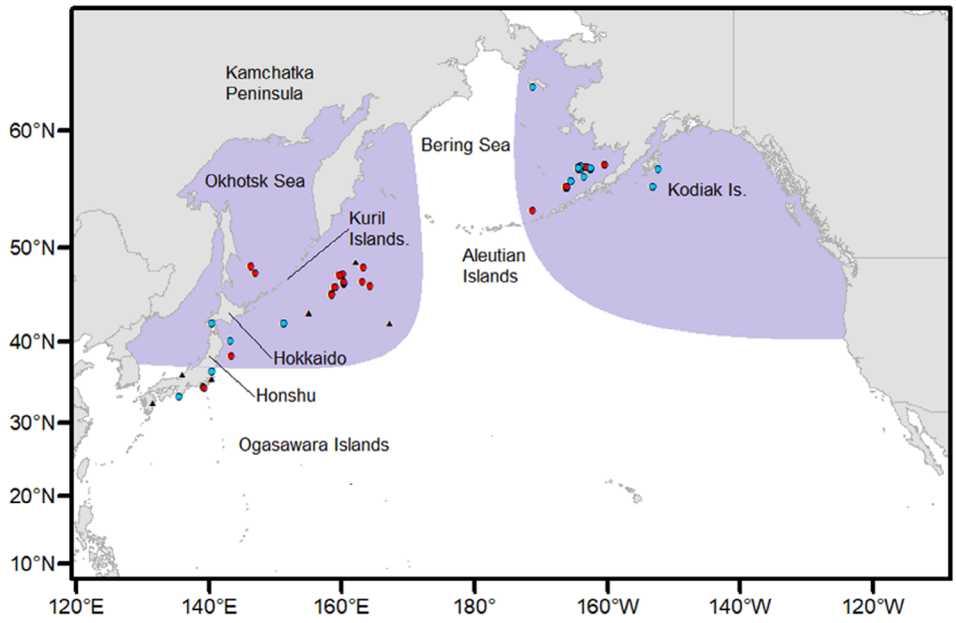

FIGURE 1 Approximate core distribution of North Pacific right whales in summer (July–August) based on 19th century whaling records and 20th century sighting and whaling catch data compiled by Clapham et al. (2004). The pattern of distribution shows high density in the western and eastern sides of the North Pacific (purple) and low density in the central area (white). The monthly plots of right whale sightings and catches by Clapham et al. (2004) suggested a pattern of south–north migratory movement in spring (March–May) to summer (June–August) on both sides of the North Pacific. The figure also shows the distribution of genetic samples of the species examined in the present study. Most of the samples from the eastern side are from LeDuc et al. (2012).

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' in the Gulf of Alaska." Gilmore (1956) proposed 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, stranding, and sighting distribution have been examined thoroughly, and the patterns have been interpreted in the context of population structure across the North Pacific (Brownell et al., 2001; Clapham et al., 2004; Scarff, 1986, 1991). For example, Brownell et al. (2001) examined all available 20th century records of right whales in the North Pacific, including sightings, strandings, 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, one in the western North Pacific and another in the eastern North Pacific. Clapham et al. (2004) provided monthly plots of right whale sightings 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 that two largely discrete populations exist on both sides of the North Pacific. Figure 1 shows the approximate core distribution of right whales in the North Pacific in July and August based on data compiled by Clapham et al. (2004). Furthermore, right whales in the eastern and western North Pacific appear to have distinct catch and recovery histories, further supporting the idea that at least two populations exist in the North Pacific (Brownell et al., 2001).

LeDuc et al. (2012) conducted the only genetic study focused on North Pacific right whale's population structure and diversity to date, but the study was based almost entirely on eastern North Pacific samples. Among 49 biopsy

samples examined (including 47 from the southeastern Bering Sea, one from the Gulf of Alaska, and one from eastern Kamchatka Peninsula), the authors identified 24 individual whales (23 from the eastern North Pacific 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 was the relationship of the eastern North Pacific right whales to those in the western North Pacific.

The main objective of this study was to test the hypothesis that two populations exist by analyzing genetic samples of right whales from the eastern and western North Pacific. The comparative genetic analyses were based on mitochondrial DNA (mtDNA) control region sequences. Microsatellite DNA data were available only for a subset of the samples from the western North Pacific, and these data were used to estimate the degree of nuclear DNA diversity in whales on the western side of the North Pacific.

Table 1 shows the list of samples representing individual North Pacific right whales that were used in the present study. Most of the eastern North Pacific (ENP) samples were from the previous study of LeDuc et al. (2012). Mitochondrial DNA sequences from historical baleen plate samples (two in the western North Pacific (WNP) and three in the ENP) were available from Rosenbaum et al. (2000). The new samples come mainly from the WNP, and they were from different sources: bycaught and stranded whales on Japan's coast, and biopsies obtained using a Larsen system (Larsen, 1998) during Japanese dedicated sighting surveys. For the ENP, six new biopsy samples obtained using the Larsen system during the International Whaling Commission-Pacific Ocean Whale and Ecosystem Research program (IWC-POWER) were available for the present study. Contemporary samples were collected in the WNP between 2003 and 2018, and in the ENP between 1997 and 2018 (Table 1).

Genomic DNA was extracted from approximately 0.05 g of the outer epidermal layer of the skin tissue using standard phenol-chloroform protocols (Sambrook et al., 1989) or using Gentra Puregene kits (QIAGEN). Extracted DNA was stored in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). The first 470 base pairs (bp) at the 5′ end of the mtDNA control region were amplified by polymerase chain reaction using primers MT4 (Árnason et al., 1993) and Dlp5R (5′-CCA-TCG-AGA-TGT-CTT-ATT-TAA-GGG-GAA-C-3′). Reactions were carried out in 25 μl volumes containing 10–100 ng of DNA, 0.1 M of each primer, 0.5 units of Ex Taq DNA polymerase (Takara), 0.2 mM of each dNTP, and 1× Ex Taq buffer (Mg^{2+} plus) (Takara). After an initial denaturation step at 95°C for 5 min, a PCR amplification cycle of 30 s at 94°C, 30 s at 50°C, and 30 s at 72°C was repeated 30 times. The amplification was completed with a final extension step of 10 min at 72°C. PCR products were purified using MicroSpin S-400HR columns (Pharmacia Biotech). Cycle sequencing reactions were performed with 1/10 volume of products generated in the above PCR amplifications using a BigDye Terminator Cycle Sequencing Kit, ver. 3.1 (Applied Biosystems) and the same primers employed in the initial PCR amplification. The cycle sequencing products were purified using AutoSeq G-50 spin columns (Pharmacia Biotech). A total of 25 cycles for 10 s at 96°C, 20 s at 56°C, and 4 min at 60°C was performed. Sequencing reaction products were size-separated and visualized on an Applied Biosystems 3500 Genetic Analyzer (Life Technology) under standard conditions. Each sample was sequenced in both forward and reverse directions (i.e., using both primers). The program Sequence Navigator was used to edit the sequences and put the forward and reverse together into a consensus sequence. Cases of singletons (haplotypes found in a single individual) were confirmed by repeating the sequencing (in both directions) for the particular samples.

Genetic variation at the nuclear level was examined at 13 microsatellite loci: EV1*Pm*, EV14*Pm*, EV21*Pm*, EV37*Mn*, EV94*Mn* (Valsecchi & Amos, 1996), GT023, GT211, GT310 (Bérubé et al., 2000), GATA028 (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 GT023 (instead of the locus GATA417 used in the original publication), males showed amplified products of both SRY and GT23 loci, while females showed only GT023. Size fractionation was done on the 3500 Genetic Analyzer (Table 2).

Although locus amplification largely followed those of the original authors, the annealing temperature for each locus was optimized for the species (Table 2). Four multiplex and five single PCR amplifications (Table 2) were performed in 15 μl reaction mixtures containing 10–100 ng of DNA, 0.25 μM of each primer, 0.35 units of Ex Taq DNA

TABLE 1 List of individual North Pacific right whales used in this study, including oceanic basin, sampling source, date, and location. There are four cases of mother and calf pairs sampled at the same time and location, as denoted by the suffix "-M" and "-C" in the sample ID column.

Oceanic basin	Sample ID	Source	Date	Sampling locality	Sex	Reference
WNP	03BC034	Bycatch	April 1, 2003	33°28.3′N, 135°50.3′E	M	Present study
WNP	11BC025	Bycatch	February 27, 2011	32°46.6′N, 131°56.4′E	—	Present study
WNP	16BC119	Bycatch	October 16, 2016	42°07.2′N, 140°37.2′E	M	Present study
WNP	18BC029	Bycatch	February 18, 2018	34°57.6′N, 139°08.8′E	M	Present study
WNP	BJM456	Stranding	April 1, 2003	36°39.4′N, 140°42.2′E	M	Present study
WNP	BJM738	Stranding	February 21, 2005	34°45.0′N, 139°21.0′E	F	Present study
WNP	BJM1067	Stranding	January 28, 2007	36°07.0′N, 136°03.0′E	—	Present study
WNP	BJM1408	Stranding	March 26, 2009	35°46.0′N, 140°49.0′E	—	Present study
WNP	B03NPRI01	Biopsy	July 31, 2003	42°12.6′N, 167°25.3′E	—	Present study
WNP	B04NPRI01	Biopsy	August 4, 2004	48°53.8′N, 162°14.2′E	—	Present study
WNP	B05NPRI01-M	Biopsy	July 23, 2005	43°17.3′N, 155°05.3′E	—	Present study
WNP	B05NPRI02-C	Biopsy	July 23, 2005	43°17.3′N, 155°05.3′E	—	Present study
WNP	11ENPSRI01	Biopsy	May 19, 2011	42°03.6′N, 151°21.5′E	F	Present study
WNP	11ENPSRI02	Biopsy	May 19, 2011	42°10.1′N, 151°23.7′E	M	Present study
WNP	11NPSRI01	Biopsy	May 21, 2011	48°03.0′N, 163°27.7′E	F	Present study
WNP	11NPSRI02	Biopsy	May 21, 2011	46°51.8′N, 163°08.1′E	F	Present study
WNP	11NPSRI03	Biopsy	May 27, 2011	46°37.7′N, 160°34.1′E	F	Present study
WNP	11NPSRI04	Biopsy	May 27, 2011	46°40.8′N, 160°32.7′E	M	Present study
WNP	11NPSRI05	Biopsy	May 27, 2011	46°56.9′N, 160°25.6′E	F	Present study
WNP	11NPSRI06-M	Biopsy	May 28, 2011	47°34.6′N, 160°08.9′E	F	Present study
WNP	11NPSRI07-C	Biopsy	May 28, 2011	47°34.6′N, 160°08.9′E	F	Present study
WNP	11NPSRI08	Biopsy	May 30, 2011	46°02.8′N, 159°04.6′E	F	Present study
WNP	11NPSRI09	Biopsy	May 30, 2011	46°02.8′N, 159°04.6′E	M	Present study
WNP	11NPSRI10	Biopsy	May 30, 2011	46°02.8′N, 159°04.6′E	F	Present study
WNP	11NPSRI11	Biopsy	May 30, 2011	45°31.5′N, 158°51.0′E	M	Present study
WNP	11NPSRI12	Biopsy	May 30, 2011	45°17.9′N, 158°45.2′E	F	Present study
WNP	B12NPRI01	Biopsy	June 6, 2012	40°06.5′N, 143°17.8′E	M	Present study
WNP	B15NPRI01	Biopsy	April 29, 2015	47°20.5′N, 159°58.3′E	F	Present study
WNP	B15NPRI02	Biopsy	May 9, 2015	46°12.0′N, 164°24.1′E	F	Present study
WNP	NPBa2	Baleen	May 23, 1956	38°32.0′N, 143°40.0′E	F	Rosenbaum et al., 2000
WNP	NPBa1	Baleen	July 25, 1968	48°14.0′N, 146°39.0′E	F	Rosenbaum et al., 2000
WNP	53766	Biopsy	August 5, 2003	47°46.0′N, 147°06.0′E	F	LeDuc et al., 2012
ENP	NPBa4	Baleen	August 22, 1961	55°53.0′N, 153°06.0′W	M	Rosenbaum et al., 2000
ENP	NPBa5	Baleen	August 22, 1961	55°54.0′N, 153°07.0′W	M	Rosenbaum et al., 2000
ENP	NPBa3	Baleen	July 30, 1962	53°42.0′N, 171°17.0′W	F	Rosenbaum et al., 2000
ENP	17081016	Biopsy	August 6, 2017	57°36.5′N, 160°43.5′W	M	Present study
ENP	17081019	Biopsy	August 8, 2017	57°37.7′N, 160°29.5′W	F	Present study
ENP	17081020	Biopsy	August 9, 2017	57°21.8′N, 163°06.5′W	M	Present study
ENP	18081004	Biopsy	July 18, 2018	57°03.2′N, 162°47.8′W	M	Present study

TABLE 1 (Continued)

Oceanic basin	Sample ID	Source	Date	Sampling locality	Sex	Reference
ENP	18081005	Biopsy	July 18, 2018	57°03.6′N, 162°47.8′W	M	Present study
ENP	18081014	Biopsy	July 26, 2018	63°14.8′N, 171°14.7′W	M	Present study
ENP	7965	Biopsy	July 20, 1997	57°08.0′N, 162°50.0′W	M	LeDuc et al., 2012
ENP	43860	Biopsy	September 8, 2004	55°52.0′N, 166°14.0′W	M	LeDuc et al., 2012
ENP	43849-M	Biopsy	September 7, 2004	56°01.0′N, 165°54.0′W	F	LeDuc et al., 2012
ENP	43850-C	Biopsy	September 7, 2004	56°01.0′N, 165°54.0′W	M	LeDuc et al., 2012
ENP	43871	Biopsy	September 9, 2004	55°48.0′N, 166°11.0′W		LeDuc et al., 2012
ENP	43851	Biopsy	September 7, 2004	56°01.0′N, 165°54.0′W	M	LeDuc et al., 2012
ENP	43858	Biopsy	September 8, 2004	55°52.0′N, 166°14.0′W	F	LeDuc et al., 2012
ENP	43867-M	Biopsy	September 9, 2004	55°48.0′N, 166°19.0′W	F	LeDuc et al., 2012
ENP	43866-C	Biopsy	September 9, 2004	55°48.0′N, 166°19.0′W	M	LeDuc et al., 2012
ENP	28432	Biopsy	August 28, 2002	56°35.0′N, 163°52.0′W	M	LeDuc et al., 2012
ENP	28426	Biopsy	August 25, 2002	57°15.0′N, 164°30.0′W	M	LeDuc et al., 2012
ENP	13192	Biopsy	July 11, 1999	57°05.0′N, 164°04.0′W	M	LeDuc et al., 2012
ENP	28424	Biopsy	August 24, 2002	57°02.0′N, 164°25.0′W	F	LeDuc et al., 2012
ENP	13190	Biopsy	July 11, 1999	57°05.0′N, 164°04.0′W	M	LeDuc et al., 2012
ENP	7966	Biopsy	July 20, 1997	57°08.0′N, 162°50.0′W	M	LeDuc et al., 2012
ENP	124913	Biopsy	August 14, 2009	57°15.6′N, 163°39.0′W	F	LeDuc et al., 2012
ENP	43853	Biopsy	September 8, 2004	55°52.0′N, 166°14.0′W	M	LeDuc et al., 2012
ENP	28430	Biopsy	August 27, 2002	57°24.0′N, 164°06.0′W	M	LeDuc et al., 2012
ENP	28427	Biopsy	August 25, 2002	57°15.0′N, 164°30.0′W	M	LeDuc et al., 2012
ENP	28428	Biopsy	August 25, 2002	57°09.0′N, 164°34.0′W	M	LeDuc et al., 2012
ENP	43865	Biopsy	September 9, 2004	55°48.0′N, 166°19.0′W	M	LeDuc et al., 2012
ENP	43856	Biopsy	September 8, 2004	55°52.0′N, 166°14.0′W	F	LeDuc et al., 2012
ENP	62934	Biopsy	August 6, 2005	57°01.0′N, 152°37.0′W	M	LeDuc et al., 2012

polymerase (Takara), and 0.2 mM of dNTPs, and 1x Ex Taq buffer (Mg^{2+} plus) (Takara). All PCR products were electrophoresed on an Applied Biosystems 3500 Genetic Analyzer. Loading details on the 3500 Genetic Analyzer of the PCR amplifications are shown in Table 2. Allele sizes were determined using a GS-600 LIZ size standard and GeneMapper v. 5.0 (ABI) software.

MtDNA sequences from this study, from LeDuc et al. (2012), and from historical baleen plates samples from WNP ($n = 2$) and ENP ($n = 3$) (Rosenbaum et al., 2000) were combined to produce a single data set. Sequences were aligned by eye using Sequence Navigator (ABI). Variable sites and unique sequences (haplotypes) were identified using the program MacClade ver. 4 (Maddison & Maddison, 1989). The degree of mtDNA intrapopulation diversity was estimated using the number of haplotypes and nucleotide and haplotype diversities (Nei, 1987) using the computer program ARLEQUIN ver. 3.5 (Excoffier & Lischer, 2010). The degree of mtDNA divergence between WNP and ENP whales was quantified by estimating conventional F_{ST} using the program ARLEQUIN ver. 3.5. Statistical significance was evaluated using 10,000 Monte Carlo simulations. A test for heterogeneity of haplotype frequencies between WNP and ENP right whales was conducted by the randomized chi-square test (Roff & Bentzen, 1989) using the R package *stats* (R Development Core Team, 2004). 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. To infer phylogenetic relationships among the mtDNA control region haplotypes, a statistical

parsimony network (Clement et al., 2000) of all haplotypes was constructed using the program PopART (Leigh & Bryant, 2015).

The computer program MICRO-CHECKER (van Oosterhout et al., 2004) was used to check for null alleles and reading/typing errors for the microsatellite data. Furthermore, a blind test was conducted by repeating the genotyping of all loci for 15 out of 19 samples to estimate genotyping errors. Genotypic linkage disequilibrium (LD) test for each pair loci was performed using GENEPOP ver. 4.0 (Rousset, 2008) under the program's default setting. The number of alleles per locus, inbreeding coefficient (F_{IS}), observed (H_O), and expected (H_E) heterozygosities per locus were calculated using FSTAT 2.9.3 (Goudet, 1995). Statistical tests for deviations from expected Hardy–Weinberg genotypic proportions were conducted using GENEPOP under the program's default setting, and this was done by each locus as well as for all loci combined. To correct *p*-values for multiple comparisons while maintaining a low false positive rate, the false discovery rate (FDR) correction (Benjamini & Yekutieli, 2001) was used.

In the WNP, different samples obtained from the same group and exhibiting the same genotypes, haplotypes and sex were considered as duplicates (i.e., samples that were collected from the same individual). Except for the duplicates, all samples differed from each other at five or more loci. In the ENP, duplicates were removed from the LeDuc et al. (2012) data prior to its inclusion in this study (see supplemental material in Wade et al., 2011, for details). After removing duplicates, the 32 samples from the ENP (23 samples from LeDuc et al., 2012, three historical baleen samples, and six new samples in this study) contained two cases of known mother and calf pairs that were sampled while affiliated. This deduction was based initially on field observation, e.g., based on body size or association with a larger whale, and confirmed by mtDNA analysis (same haplotype) and by genotyping data when available (sharing an allele at every locus). The 32 samples from the WNP (29 samples from this study, one from LeDuc et al., 2012, and two historical baleen samples) contained two cases of known mother and calf pairs that were sampled while together. Seven WNP samples failed to sex. The proportion of males was 0.36 ($n = 25$ with sex data available) and 0.75 ($n = 32$) for the WNP and ENP, respectively. The sex determination of the historical samples (baleen) was three females and two males (Table 1). The rest of the statistical analyses were conducted after removing one sample from each mother and calf pair, resulting in sample sizes for the mtDNA statistical analyses of 30 in each the WNP and ENP, respectively (Table 1, Figure 1); the sample size for the microsatellite analysis was 19 for the WNP. All these samples were successfully genotyped at all loci.

Regarding microsatellite DNA, no evidence of null alleles was found. Also, no evidence of genotypic linkage disequilibrium for each pair loci was found. The results of the blind test indicated genotyping error due to overlapping peaks between the two alleles for one locus (GT310) in three of the 15 samples tested. These three samples were homozygous for this locus in the first genotyping (103/103) but heterozygous in the second genotyping (101/103). For the whales in the WNP, the number of alleles per locus ranged from 2 to 7 (4.08 on average), and the H_E ranged from 0.285 to 0.787 (0.593 on average). The F_{IS} in each locus ranged from −0.045 to 0.350 with 0.032 on average. After correcting for multiple comparisons, we found no significant deviations from the Hardy–Weinberg genotypic proportion across loci ($\chi^2 = 33.15$, $df = 26.00$, $p = .158$; Table 2). GT310 was the only locus deviating significantly from the Hardy–Weinberg equilibrium, and with a positive F_{IS} value, suggesting an excess of homozygosity (Table 2). This result was most likely produced by genotyping error found at this locus. Values of microsatellite DNA diversity did not change substantially when this locus was excluded from the analyses (data not shown). The average expected heterozygosity in the WNP population (0.593) is almost double the level of diversity estimated for the critically endangered North Atlantic right whale (*Eubalaena glacialis*) ($H_E = 0.37$; Waldick et al., 2002), and smaller than that estimated for the southern right whales (*Eubalaena australis*; $H_E = 0.77$; Carroll et al., 2019). However, these estimates are based on different sets of microsatellite loci and thus are not directly comparable. The microsatellite DNA genotypes will be available to interested scientists following requests to the corresponding author.

The final data set included the first 399 bp of the mtDNA control region. A total of 16 haplotypes were identified among 60 individual whales. This number was derived from 25 transitions and one insertion/deletion (Table 3). The sequences of the six new haplotypes have been deposited in GenBank under accession numbers OL574295-OL574300. Table 4 shows the indices of mtDNA diversity in North Pacific right whales in comparison

TABLE 2 Indices of microsatellite DNA diversity in western North Pacific right whales: *n*, number of samples; A, number of alleles; F_{IS}, inbreeding coefficient; H_O, observed heterozygosity; H_E, expected heterozygosity; HW, *p*-value for the test of Hardy–Weinberg equilibrium; M, multiplex PCR amplification; S, single PCR amplification; Seq, combination of PCR products co-loaded in sequencing; Temp, annealing temperature.

Microsatellite loci	PCR	Seq	Temp	*n*	A	F_{IS}	H_O	H_E	HW
EV1*Pm*	M1	Sq1	58	19	5	0.093	0.684	0.752	0.971
GT310									
GT023	M2	Sq2	58	19	5	−0.125	0.842	0.751	0.932
EV94*Mn*									
EV14*Pm*	M3	Sq3	54	19	4	0.119	0.421	0.477	0.132
GT211									
EV37*Mn*	S1	Sq1	54	19	5	0.065	0.737	0.787	0.210
GATA028									
EV21*Pm*	M4	Sq5	54	19	2	−0.241	0.632	0.512	0.378
DlrFCB17									
TR3G2	S3	Sq6	50	19	5	−0.157	0.842	0.731	0.630
TR2G5									
TR3F2	S5	Sq7	58	19	3	−0.146	0.368	0.323	1.000
Overall				19	4.08		0.575	0.593	0.158

with those in North Atlantic and southern right whales. In the North Pacific, both nucleotide and haplotype diversity indices indicated a relatively high diversity with both indices slightly lower in the ENP right whales. For the same sample size, the numbers of singletons (i.e., haplotypes found in only a single individual) were higher in the WNP whales. At the worldwide level, the lowest haplotype and nucleotide diversities were observed in North Atlantic right whales. The level of diversity in North Pacific right whales was, in general, intermediate between western North Atlantic and Southern Hemisphere populations, which was more evident for the case of nucleotide diversity (Table 4).

In the North Pacific, there was no concordance between geography and haplotype network pattern (Figure 2). 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 also explain the relatively high level of haplotype and nucleotide diversities in both populations (see Table 4) despite the 19th century high whaling pressure.

Only four haplotypes were shared between WNP and ENP right whales (Table 3). Eight haplotypes involving 21 individuals were specific to the WNP, whereas four haplotypes involving 19 individuals were specific to the ENP. The F_{ST} between WNP and ENP right whales was high (0.128) and statistically significant ($p < .001$), and the heterogeneity test based on the randomized chi-square test resulted in significant statistical differences between whales on both sides of the North Pacific ($\chi^2 = 42.91$, $p < .001$). The F_{ST} between WNP and ENP right whales was similar to those estimated between North Pacific and North Atlantic common minke whales (*Balaenoptera acutorostrata*) (0.117) (Milmann et al., 2021) and humpback whales (*Megaptera novaeangliae*) (0.180) (Jackson et al., 2014). The results of the mtDNA analysis therefore showed striking genetic differentiation between WNP and ENP right whales. Additional analyses involving sequencing the full mitogenome might provide additional phylogenetic resolution, as has been seen in killer whales, *Orcinus orca*, (Morin et al., 2010) and fin whales (Archer et al., 2013).

These mtDNA results, suggesting some degree of population structuring, are consistent with the pattern of catch and sighting data showing higher densities on either side of the North Pacific, but little in between (Clapham et al., 2004) (Figure 1). Therefore, the pattern of historical catches, the sighting distribution, and the genetic data

TABLE 3 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. Nucleotide position 1 corresponds to position 15,468 in the full control region sequence of North Pacific right whale with accession number NC_006931. Asterisks refers to haplotype/samples of historical baleen plate samples used in a previous study. Single asterisks: haplotypes 2 and 6 (LeDuc et al., 2012) are the same haplotype as AF275357 and AF275356, respectively (Rosenbaum et al., 2000); double asterisks: same individuals as those reported in Rosenbaum et al. (2000) even though the sequence lengths are different between the two studies, 399 bp in the present study and 287 bp in the previous study.

	Nucleotide position of the polymorphic sites			Frequency			
Haplotype ID	11 266789900 6448965604	1111122222 1244802488 1634868645	223333 891225 623124	Western North Pacific	Eastern North Pacific	Total	Accession No.
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	12	12	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	AF275352 (Rosenbaum et al., 2000)**
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	4	0	4	AF275355 (Rosenbaum et al., 2000)**
10	. . . . C . . C . G	A . T . ACT . G .	. . . . .	2	0	2	OL574295 (Present study)
11	. . . . C . . . C .	. . . . A . T . . .	C . G . . T	1	0	1	OL574296 (Present study)
12	. . . . . C . .	A . . . ACT . . .	CCG . . .	1	0	1	OL574297 (Present study)
13	. . . . . G	. . . . A . T . . .	. . . GA .	1	0	1	OL574298 (Present study)
14	. . . . . G	. . . . A . T . . .	. . . G . .	3	0	3	AF275354 (Rosenbaum et al., 2000)**
15	. . . C . . . C . .	. . . CACT . G .	. . . . .	3	1	4	OL574299 (Present study)
16	. . T. CT . . CG	. . . . A . T . . .	CC	1	0	1	OL574300 (Present study)
Total				30	30	60	

based on mtDNA support the hypothesis of different populations occurring in the eastern and western sides of the North Pacific. While the migration pattern of these two populations has been described by several authors (Clapham et al., 2004; Gilmore 1956; Omura 1958; Omura et al., 1969), 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. Based on the pattern of catches around Japan, Omura (1986) further suggested the occurrence of two populations in the waters surrounding the Japanese Archipelago, one wintering in the Sea of Japan/East China

TABLE 4 Indices of mtDNA diversity in North Pacific right whales in comparison with those in North Atlantic and southern right whales. WNP = western North Pacific; ENP = eastern North Pacific; WNA = western North Atlantic; SH = Southern Hemisphere; ARG = Argentinian nursery ground; SA = South African nursery ground; NZ = New Zealand nursery ground; SWA = southwest Australian nursery ground. SH-Antarctic refers to IWC management Area IV (70°–130°E). Other symbols are as follows: n = sample size; bp = length of the mtDNA control region sequence; n_{hap} = number of haplotypes; h = haplotype diversity; SD = sample standard deviation; π = nucleotide diversity.

Oceanic basin	n	bp	n_{hap}	h (SD)	π (SD) %	Reference
WNP	30	399	12	0.89 (0.03)	1.75 (0.94)	This study
ENP	30	399	8	0.80 (0.06)	1.63 (0.88)	This study
WNA	269	500	5	0.70 (0.02)	0.60 (0.30)	Malik et al. (1999); Rosenbaum et al. (2000)
SH-ARG	208	500	28	0.94 (0.01)	2.27 (1.16)	Carroll et al. (2019)
SH-SA	350	500	37	0.94 (0.01)	2.47 (1.26)	Carroll et al. (2019)
SH-NZ	692	500	11	0.69 (0.01)	1.49 (0.79)	Carroll et al. (2019)
SH-SWA	16	500	5	0.71 (0.09)	1.79 (1.00)	Carroll et al. (2019)
SH-Antarctic	67	275	10	0.77 (0.03)	2.34 (0.09)	Pastene et al. (2016)

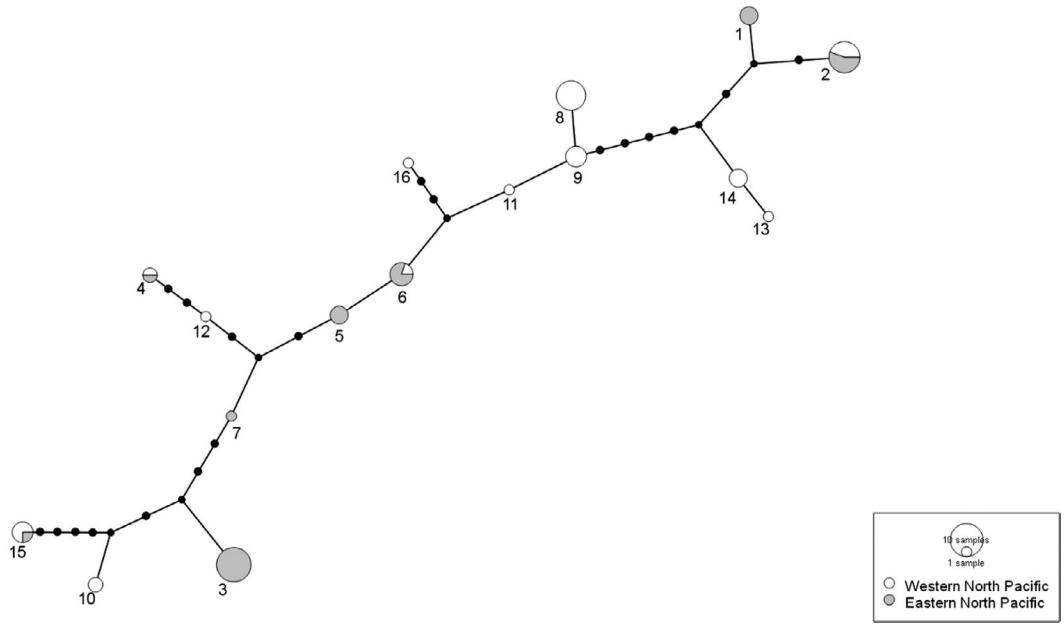

FIGURE 2 Statistical parsimony network based on 16 mtDNA haplotypes of North Pacific right whales. Each line and circle indicate a single mutational step and haplotype, respectively. Circle size refers to haplotype abundance and populations are shaded: dark gray, eastern North Pacific; white, western North Pacific. Small black filled circles indicate intermediate haplotypes not found in this study.

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. Only one genetic sample was available for this study from a right whale from the Sea of Japan, and that whale exhibited haplotype 2, which was the second most common haplotype occurring in both western and eastern populations.

An alternative interpretation of the genetic results in the present study is that there is a single interbreeding population in the North Pacific that is exhibiting mtDNA structuring as a result of matrilineally driven seasonal site fidelity. Addressing this possibility would require that nuclear data be compared between ENP and WNP sample sets; given that only one of the microsatellite loci genotyped in this study was also genotyped in the LeDuc et al. (2012), such a comparison would require the generation of additional data. However, while this alternative interpretation cannot be ruled out given the existing genetic data sets, the data on catches and sightings are consistent with north–south migratory movements and thus support the hypothesis of two largely discrete breeding populations of right whales in the eastern and western North Pacific (Brownell et al. 2001, Clapham et al. 2004). In addition, LeDuc et al. (2012) estimated that the genetic effective size of the ENP whales is extremely low ($N_e = 11.6$, 95% CI [2.9, 75.0]) and consistent with mark-recapture estimates of the total abundance of whales using the southeastern Bering Sea shelf and the Aleutian Islands (~30 whales; Wade et al. 2011). For the WNP, the abundance was estimated 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 (these estimates have not been reviewed by the IWC Scientific Committee; Hakamada & Matsuoka, 2016). Given that the estimated abundance of whales in the WNP is an order of magnitude or more, greater than that estimated for the ENP, the calculated N_e for the ENP would be expected to be larger if substantial interbreeding with the WNP whales was occurring.

In summary, the results of the present mtDNA analyses are consistent with those of the analyses of sighting, strandings, and catch distribution that suggested different populations on both sides of the North Pacific. The smaller number of haplotypes and singletons as well a smaller proportion of females in comparison with the western population confirm the critical situation of the eastern right whale population. Regardless of the need for additional analyses, particularly evaluating genetic differences between populations using nuclear markers, the current available genetic and nongenetic information suggests that WNP and ENP right whales require separate management for their conservation.

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AUTHOR CONTRIBUTIONS

Luis Pastene: Conceptualization; formal analysis; investigation; project administration; supervision; writing – original draft; writing – review and editing. **Mioko Taguchi:** Data curation; formal analysis; investigation; methodology. **Aimee Lang:** Conceptualization; data curation; investigation; methodology; validation; writing – review and editing. **Mutsuo Goto:** Data curation; formal analysis; investigation; methodology. **Koji Matsuoka:** Data curation; investigation; methodology.

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