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Abstract

We report full mitogenome sequences for 74 North Pacific gray whales including 38 western gray whales sampled at their summer feeding grounds near Sakhalin Island and 36 eastern gray whales sampled at their wintering breeding grounds in Mexico. The relationship of these two populations is uncertain since recent studies have shown at least some western gray whales to winter in Mexico. Haplotype diversity was much higher in the large (ca. 27,000) eastern gray whale population compared to the small western gray whale population (200-300) but no phylogeographic structure is evident in the data. This is suggestive of female mediated gene flow or a recent divergence of the populations. Historical demographic analysis shows the North Pacific gray whales experienced a population bottleneck that predated the Last Glacial Maximum (LGM) but fails to resolve an expected recent population bottleneck caused by commercial whaling. Mitochondrial diversity was likely already reduced at the time of whaling.

Introduction

Anthropogenic change is rapidly altering marine ecosystems with unknown consequences for the marine biota, including marine mammals (Doney *et al.* 2012; Jackson 2008). These changes are both direct (e.g., commercial whaling, ship strikes) and indirect (e.g., ocean warming, pollution, industrial development). We are just beginning to understand how marine mammals responded to past environmental perturbations like the Pleistocene glacial periods (Phillips *et al.* 2011), which is important for conservation efforts as it enables us to make informed predictions on how they may respond to the ongoing environmental changes (Kaschner *et al.* 2011; Parmesan & Yohe 2003; Ramp *et al.* 2015). Many populations of great whales (Mysticeti) are especially vulnerable after being decimated by unregulated commercial whaling in the 19th and 20th centuries leading to very low population sizes for the majority of great whales (Baker & Clapham 2004; Roman & Palumbi 2003).

The gray whale (*Eschrichtius robustus*) is one species that has already been impacted by both anthropogenic and climate changes (Alter *et al.* 2015; Árnason *et al.* 2018; Brüniche-Olsen *et al.* 2018b). Since disappearing from the Atlantic Ocean due to whaling, it is now found only in the North Pacific Ocean (Alter *et al.* 2015). There are two mixed-stock populations, with some degree of gene flow between them (Alter *et al.* 2015; Brüniche-Olsen *et al.* 2018a; DeWoody *et al.* 2017; Lang *et al.* 2010; LeDuc *et al.* 2002). The eastern gray whale numbers about 27,000 and migrates along the Pacific coast of North America from wintering breeding grounds in Mexico to summer feeding grounds primarily in the Bering Sea (Figure 1), making it among the longest migrating mammalian species in the world (i.e., >20,000 km annually, Mate *et al.* 2015). The western gray whale numbers only about 200 to 300 individuals and migrates along the Asian coast from unknown wintering breeding grounds probably in the South China Sea to summer feeding grounds in the Sea of Okhotsk. Today this small population summers primarily off the northeastern coast of Sakhalin Island, Russia (Figure 1). This population was presumed extinct due to whaling, but was re-discovered in the 1980s. Uncertainty regarding the affiliation of the western gray whales was raised when satellite transmitters were placed on three whales that migrated from Sakhalin to or towards North America and the eastern gray whale wintering grounds in Mexico (Mate *et al.* 2015).

Whether gray whales may benefit from climate warming is a complex question. Environmental niche modelling of future gray whale habitat for year 2100 indicates expanded habitat with future climate warming (Brüniche-Olsen *et al.* 2018b), and decreased ice cover on the feeding grounds is correlated with a longer feeding season for reproductive females, and a resulting higher calf survival to the following year (Gailey *et al.* 2020). However, other factors might negate such a benefit. The effects of climate warming on food resources and distribution, increased ship traffic in the Arctic, hydrocarbon development, and changes in commercial fishing practices could have detrimental impacts on gray whale survival (Coyle *et al.* 2007; Reeves *et al.* 2014). Despite the challenges to survival of the endangered western gray whales, the population has increased at an annual rate of 4 to 5% between the years 1995 to 2015 (Cooke, 2018; Cooke *et al.* 2019), yet it remains a small population.

The erosion of genetic diversity is well known as a factor that contributes to increased probability of extinction. Conservation geneticists routinely use mtDNA to test for genetic bottlenecks and to reconstruct the deep historical demography of populations. However, detection of very recent bottlenecks for long-lived species like baleen whales (Taylor *et al.* 2007) is technically very difficult unless genetic samples of the population pre-bottleneck are available (Leonard 2008). In eastern gray whales pre- and post-whaling genetic samples have been used to assess impacts of commercial whaling. The results illustrate the large uncertainty associated with inferring recent demographics. In one study eastern gray whales showed no change in diversity for mtDNA (~450bp) and microsatellites (Béland et al. 2019). In contrast other studies have identified a recent bottleneck based on mtDNA (383bp) (Alter *et al.* 2012), and microsatellite data estimated the eastern population to be at 28–56% of its pre-whaling abundance (Alter *et al.* 2007). No recent bottleneck estimate for the western gray whales is available, but it is clear that the this population was even more greatly impacted by commercial whaling than the eastern gray whale (Swartz *et al.* 2006).

In this study, we use complete mitochondrial genomes from 70+ eastern and western gray whales to: i) assess population structure and gene flow between the populations, and ii) reconstruct the female demographic history to determine past population dynamics. This information will be useful for other studies of recent population dynamics for long lived species like great whales and inform gray whales conservation efforts.

Methods

Tissue collection and mitogenome sequencing

We extracted DNA from tissue biopsy samples from 69 gray whales from the eastern and western Pacific (Figure 1). The individuals used herein were previously genotyped at 91 autosomal loci (Brüniche-Olsen *et al.* 2018a). The samples were collected off Baja, Mexico and off Sakhalin Island, Russia between 2011 and 2016 using a 150 lb draw weight compound crossbow with 40mm by 7mm internal diameter tip arrows. DNA sequences were generated using a NovaSeq with paired end (PE) reads, which we used to reconstruct the entire mitochondrial genomes. We added PE reads from two Sakhalin gray whales (DeWoody *et al.* 2017) and three eastern gray whales (Árnason *et al.* 2018; DeWoody *et al.* 2017) giving a total of 74 complete mitogenomes.

For mitogenome *de novo* assembly we used NOVOPLASTY v3.1 (Dierckxsens *et al.* 2016). To facilitate circular sequence alignment we used MARS (Ayad & Pissis 2017), and realigned the sequences using MAFFT (Katoh & Standley 2013). We masked ambiguous nucleotides in the mitogenome sequences "N". The sequence alignment were inspected visually in UGENE v1.32.0 (Okonechnikov *et al.* 2012). To quality check our data we inspected known mother-offspring pairs to confirm that they had the same haplotype, and identified outlier haplotypes using delta-likelihood using PEGAS (Paradis 2010). We used ORFFINDER (Wheeler *et al.* 2003) to estimate the number of open reading frames (ORF) in the main mitochondrial clades.

Genetic diversity and population structure

We used DNAsp v5 (Librado & Rozas 2009) to calculate genetic diversity quantified as the number of haplotypes (h), haplotype diversity (h_d), the number of segregating sites (S), average number of nucleotide differences (k), and nucleotide diversity (π). Demographic changes were quantified with Fu's and Li's D^* and F^* (Fu 1997). POPART (Leigh & Bryant 2015) was used to construct a median-joining haplotype network. To assess how much of the total haplotype diversity in each population we had sampled we plotted rarefaction curves using VEGAN (Oksanen *et al.* 2010).

Phylogenetic relationships

A time-scaled phylogeny for the gray whale haplotypes was constructed using Bayesian methods. We identified The Most Recent Common Ancestor (TMRCA) lineages for the gray whale clade by constructing a phylogeny of the unique gray whale haplotypes and mitogenomes from fin whale (*Balaenoptera physalus* KC572811.1) and humpback whale (*Megaptera novaeangliae* AP006467.1) using PHYML (Guindon *et al.* 2010). Based on the phylogeny we identified two gray whale haplotypes from the two main clades of the phylogeny (individual ER-17-0248 and ER-17-0253), and these were then used for the TMRCA prior. A log-normal prior for TMRCA (mean = 80, standard deviation = 0.15) was used based on TMRCA inferences from whole genome sequences from eastern and western pacific gray whales 80ky (60 – 100ky 95% highest posterior density (HPD)) (Árnason *et al.* 2018), and from mitochondrial data from the extinct Atlantic gray whale and the extant Pacific gray whale 79ky (63 – 102ky 95% HPD) (Alter *et al.* 2015). PARTITIONFINDER v2.1.1 (Lanfear *et al.* 2017) was used to identify the most likely partitioning scheme and model of nucleotide substitution for each region, and the result was used to inform BEAST2 (Bouckaert *et al.* 2014), accounting for codon position rate variation in the coding regions (Ho & Lanfear 2010).

To assess the level of rate heterogeneity we used an uncorrelated log-normal clock using the 'constant population' tree prior. Because the mtDNA is inherited as a single locus, we used unlinked site models, linked clock model and linked tree model. We ran the MCMC for 5×10^7 iterations sampling for every 5×10^3 iteration and removing the first 10% as burn-in. Multiple runs were done and checked for convergence and equivalent sample size (ESS) values >200 in TRACER v1.7.1 (Rambaut *et al.* 2016). The clock model was assessed in TRACER based on the 'ucldStdev' parameter. A value <0.1 indicates negligible variation in substitution rate among the lineages and thus a better fit of the strict clock (Drummond & Bouckaert 2015). The haplotype phylogeny was visualized in FIGTREE v1.4.2 (Rambaut 2012).

Demographic inference

Demographic trajectories for the female effective population size (N_{ef}) was inferred using Bayesian coalescent samplers in BEAST2 (Drummond 2008). Unequal sampling effort can confound demographic reconstruction (Heller *et al.* 2013), but as we had an equal number of samples from each part of the Pacific, and no indication of population structure (see haplotype network in Figure 2), we included all 74 individuals in the demographic analysis. The setup was the same as for the phylogeny reconstruction except here we used a log normal clock and the estimated clock rate 4.0×10^{-8} bp^{-1} $year^{-1}$ (2.3×10^{-8} – 6.0×10^{-8} 95% HPD)). To identify if the gray whales had experienced changes in N_{ef} we used the 'Extended Bayesian Skyline plot' (EBSP). The rationale for using EBSP was that we were interested in the 'sum(indicators.alltrees)' parameter, which describes the number of likely population size changes in the data. Multiple runs were done for each model and checked for convergence in TRACER. To convert to effective population size (N_e) we used a generation time (g) of 18.9 years as the midpoint between 15.5 and 22.3 years (Heppell *et al.* 2000; Rice *et al.* 1971).

Results

Genetic diversity and population structure

We obtained mitogenomes from 74 gray whales representing 36 eastern gray whales and 38 western gray whales (Figure 1). The mean depth of coverage for the mitogenomes was 250x, providing a high degree of certainty for haplotype identification. Neither eastern or western gray whales showed signs of demographic changes based on D^* and F^* (Table 1). The eastern gray whales had higher number of haplotypes and higher haplotype diversity ($h = 25$ and $h_d = 0.975$) compared to the western gray whales ($h = 9$ and $h_d = 0.723$) (Table 1). We identified similar levels of control region diversity found by earlier authors (Alter *et al.* 2015; Supplementary material Table S2). Despite their reduced number of haplotypes (only about 1/3 as many as the eastern gray whales), the western gray whales had higher π and k, reflecting that the smaller number of haplotypes had a high degree of divergence.

Our haplotype network identified two main groups, both with 57 ORFs, separated by 79 mutations, a structure similar to all previous analyses of mtDNA (Figure 2; Alter *et al.* 2015). There was no pronounced mtDNA structure between the eastern and western gray whales. Eastern gray whales were found throughout the haplotype network mainly represented by single haplotypes, whereas the majority of western gray whales consisted of only two haplotypes. Rarefaction curves of sampling effort (Figure 3) suggested that future sampling of more individuals from the eastern Pacific could continue to increase the number of haplotypes ultimately identified, but increased sampling effort in the western Pacific is unlikely to reveal many more undiscovered haplotypes (Figure 3).

Phylogenetic relationships

Our phylogenetic analysis showed that an uncorrelated log-normal clock fitted our dataset better than a strict clock model (‘uclStdev’ = 0.17). Bayesian analyses estimated a divergence time between the two main mtDNA clades of ~76.5ky (55.0 – 99.5% HPD) (Figure 3). The phylogeny is based on estimates of TMRCA inferred from whole genome sequences (Árnason *et al.* 2018) and from the control region of the mtDNA (Alter *et al.* 2015), and TMRCA was marginally more recent than our estimates. The majority of the branches coalesced 20-23ky ago during the Last Glacial Maximum (LGM) potentially reflecting the LGM bottleneck (Brüniche-Olsen *et al.* 2018b). Private haplotypes were observed in both eastern and western Pacific sampling sites (Table 1; Figure 3), but private clades were found only in the eastern population. These were recent (<15ky) suggesting limited maternal population structure between eastern and western gray whales.

Demographic inference

The EBSP analysis found ‘sum(indicators.alltrees)’ parameter to have a mean =1.07 (0 – 3 95% HPD) with zero changes being the most frequent number of changes identified (0 changes ~19,000 times, one change ~11,500 times, two change ~8,000 times and three changes ~5,000 times) as the most likely demographic model. The demographic trajectory showed that the long-term population size was ~80,000 ($N_e \times g$) corresponding to a median N_e ~4,200 and N ~25,200 assuming a generation time of 18.9 years (Figure 4). A slight recent decline in population size was observed ~70,000 ($N_e \times g$) corresponding to a median N_e ~3,700 and N ~22,200, although this might be an artefact as a constant population size could not be rejected. The majority of the demographic events occur very recently (<500 years ago), depicted by the bars, suggesting that with the mitogenome dataset we quickly loose resolution as we go back in time.

Discussion

During interglacial periods many great whales experienced drastic decline in population size (Árnason *et al.* 2018), and more recently commercial whaling further reduced populations (Baker & Clapham 2004; Roman & Palumbi 2003). These sequential population reductions are a concern as genetic diversity is lost during periods of small population size. Gray whales experienced substantial Pleistocene (Brüniche-Olsen *et al.* 2018b) and Holocene (Alter *et al.* 2015; Alter *et al.* 2012; Alter *et al.* 2007) declines compromising their population sizes. Herein we examine mtDNA population dynamics of eastern and western gray whales, searching for evidence of phylogeographic structure and tracing recent demographic trajectories. We were particularly interested in whether full mitogenomes would increase the resolution compared to shorter (400 - 500bp) mitochondrial fragments.

Population structure among maternal gray whale lineages

Studies of mtDNA control region sequences, nuclear microsatellites, and SNPs have consistently revealed a low but statistically significant F_{ST} in comparisons of eastern and western gray whales samples of various origins (Brüniche-Olsen *et al.* 2018a; Brykov *et al.* 2019; Lang *et al.* 2010; LeDuc *et al.* 2002). Genic and inter-genic SNP markers established that both the eastern and western gray whales each consist of mixed stock assemblages, with one genotype predominating in whales sampled of the Mexican wintering grounds and another genotype predominating in whales sampled of the coast of Sakhalin Island (Brüniche-Olsen *et al.* 2018a). We know from photo identification (photo ID) that whales, including males and females, of both genotypes migrate from Sakhalin to Mexico (Brykov *et al.* 2019) and we know from telemetry data that whales migrate between Sakhalin and Mexico (Mate *et al.* 2015). Our data using complete mitogenomes confirm previous studies (Brykov *et al.* 2019; Lang *et al.* 2010; LeDuc *et al.* 2002), that show mtDNA haplotype lineages with no evidence of unique lineages within the western gray whale population (Figure 2 and Figure 4) as might be expected if females were strongly philopatric due to natal homing or social facilitation (FitzSimmons *et al.* 1997). If eastern and western gray whale were two separate breeding entities we would expect that they should have evolved unique haplotype lineages, given that other marine mammals with similar geographic distributions have such distinct mtDNA lineages (Phillips et al., 2011).

Population dynamics

Studies of Atlantic and Pacific gray whale populations show that there was not only gene flow within each ocean basin, but also between ocean basins (Alter *et al.* 2015). Our analyses of whole mitogenomes from Pacific gray whales support this scenario of female-mediated gene flow through time. The majority of mitochondrial diversity is found in the eastern gray whales (Table 1), and had we increased our sampling efforts we would have been more likely to recover novel haplotypes by sampling off the coast of Mexico (Figure 3). This mimics the IUCN status with the eastern gray whale being least concern whereas the western gray whale is endangered (IUCN 2018).

The deep divergence between major clades ~75kya (Figure 4) predates the LGM and the majority of the branches coalesced 20-23ky ago potentially reflecting the LGM bottleneck (Brüniche-Olsen *et al.* 2018b), suggesting that the mitochondrial diversity was already depleted prior to commercial whaling. Our estimate of the current population size fits with the estimated census size *N* ~22,200 – 25,200. We fail to recover the presumed commercial whaling bottleneck (Figure 5). Many studies have used mtDNA to reconstruct demographic history (Brüniche-Olsen *et al.* 2014; Heller *et al.* 2012; Phillips *et al.*, 2011; Shapiro *et al.* 2004), but for long-lived species the resolution for such analyses are not optimal if the bottleneck was subtle or if it was followed by fast recovery (Mourier *et al.* 2012). Furthermore the partitioning of the dataset can greatly impact the demographic inference if population structure is present (Heller *et al.* 2013), leading to questionable results (Pérez-Alvarez *et al.* 2016). Incorporating nuclear markers along with the mitochondria in analysis can help resolve some of the signals related to for example gene flow (Carroll *et al.* 2019).

Conclusion

Phylogenetic analyses of full mitogenomes of eastern and western gray whales showed no evidence of population structure, consistent with previous studies of shorter segments of mtDNA. Previous studies have found significant differences between the populations in haplotype frequencies (LeDuc *et al*., 2002; Lange *et al*., 2011) but this was not tested in our study. Our data indicate that there has been female mediated gene flow between the two populations, or a recent divergence. Deep divergence of two major mtDNA clades predating the LGM was observed. Most of the haplotype branches coalesce 20-23 ky ago which is consistent with a previously observed LGM bottleneck based on whole genome sequences (Brüniche-Olsen *et al.* 2018b). No evidence of a recent bottleneck caused by commercial whaling was observed, likely due to insufficient resolution at recent time scales. Our estimate of current population size based on mtDNA diversity is close to the current estimated census size of the North Pacific gray whale population as a whole, and the strong difference in observed haplotype diversity between eastern and western gray whales is consistent with populations of drastically different size (27,000 vs. 200-300, respectively).

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Ethical statement

Russian and Mexican field research was approved by the Ethics Committee of the National Scientific Center of Marine Biology of the Far East Branch of the Russian Academy of Science and by the Subsecretaria de Gestion Para La Protection Ambiental of the Mexican Direcion General de Vida Silvestre, respectively. U.S. research on marine mammal samples collected abroad by foreign colleagues falls under the authority of NOAA and requires a U.S. MMPA permit as well as the proper CITES permits for import and export of the samples. Russian and Mexican collaborators obtained proper research permits, which covered ethical considerations for the collection of samples. Thus, all relevant U.S. and international permits were secured for this research. National Marine Fisheries Service Office of Protected Resources' Marine Mammal Health and Stranding Response Program permit 93-1905-MA-009526. CITES permit 13US082589/9, 13RU00580, MX89451, and MX71396.

Data accessibility

Genbank accession codes: XXXX - XXXX.

References

Alter SE, Meyer M, Post K, *et al.* (2015) Climate impacts on transocean dispersal and habitat in gray whales from the Pleistocene to 2100. *Molecular Ecology* **24**, 1510-1522.

Alter SE, Newsome SD, Palumbi SR (2012) Pre-whaling genetic diversity and population ecology in eastern Pacific gray whales: Insights from ancient DNA and stable isotopes. *Plos One* **7**, e35039.

Alter SE, Rynes E, Palumbi SR (2007) DNA evidence for historic population size and past ecosystem impacts of gray whales. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 15162-15167.

Árnason Ú, Lammers F, Kumar V, Nilsson MA, Janke A (2018) Whole-genome sequencing of the blue whale and other rorquals finds signatures for introgressive gene flow. *Science Advances* **4**, eaap9873.

Ayad LAK, Pissis SP (2017) MARS: improving multiple circular sequence alignment using refined sequences. *Bmc Genomics* **18**, 86.

Baker CS, Clapham PJ (2004) Modelling the past and future of whales and whaling. *Trends in Ecology & Evolution* **19**, 365-371.

Béland SL, Frasier BA, Darling JD, Frasier TR (2019) Using pre- and postexploitation samples to assess the impact of commercial whaling on the genetic characteristics of eastern North Pacific gray and humpback whales and to compare methods used to infer historic demography. *Marine Mammal Science*.

Bouckaert RR, Drummond AJ (2017) bModelTest: Bayesian phylogenetic site model averaging and model comparison. *Bmc Evolutionary Biology* **17**, 42.

Bouckaert RR, Heled J, Kühnert D, *et al.* (2014) BEAST 2: a software platform for bayesian evolutionary analysis. *PLoS Comput Biol* **10**.

Brüniche-Olsen A, Jones ME, Austin JJ, Burridge CP, Holland BR (2014) Extensive population decline in the Tasmanian devil predates European settlement and devil facial tumour disease. *Biology Letters* **10**.

Brüniche-Olsen A, Urban JR, Vertyankin VV, *et al.* (2018a) Genetic data reveal mixed-stock aggregations of gray whales in the North Pacific Ocean. *Biology Letters* **14**.

Brüniche-Olsen A, Westerman R, Kazmierczyk Z, *et al.* (2018b) The inference of gray whale (*Eschrichtius robustus*) historical population attributes from whole-genome sequences. *Bmc Evolutionary Biology* **18**, 87.

Brykov VA, Efimove KV, Brüniche-Olsen A, DeWoody J, Bickham JW (2019) Population structure of Sakhalin gray whales (Eschrichius robustus) revealed by DNA sequences of four mtDNA genes. *Special Publications Museum of Texas Tech University* **71**.

Carroll EL, Alderman R, Bannister JL, *et al.* (2019) Incorporating non-equilibrium dynamics into demographic history inferences of a migratory marine species. *Heredity* **122**, 53-68.

Cooke, J.G. 2018. Abundance estimates for western North Pacific gray whales for use with stock structure hypotheses of the Range-wide Review of the Population Structure and Status of North Pacific gray whales. Paper SC/67b/ASI presented to the IWC Scientific Committee (unpublished). 14 pp. [Available at http://www.iwcoffice.org]

Cooke, J.G., Sychenko, O., Burdin, A.M., Weller, D.W., Bradford, A.L., Lang, A.R., and Brownell, R.L. Jr. 2019. Population Assessment Update for Sakhalin Gray Whales. Paper SC/68A/CMP/21 presented to the IWC Scientific Committee (unpublished). 9 pp. [Available at http://www.iwcoffice.org]

Coyle KO, Bluhm B, Konar B, Blanchard A, Highsmith RC (2007) Amphipod prey of gray whales in the northern Bering Sea: comparison of biomass and distribution between the 1980s and 2002–2003. *Deep Sea Research Part II: Topical Studies in Oceanography* **54**, 2906-2918.

DeWoody JA, Fernandez NB, Brüniche-Olsen A, *et al.* (2017) Characterization of the gray whale Eschrichtius robustus genome and a genotyping array based on single-nucleotidepolymorphisms in candidate genes. *Biol Bull* **232**, 186-197.

Dierckxsens N, Mardulyn P, Smits G (2016) NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic Acids Research* **45**, e18-e18.

Doney SC, Ruckelshaus M, Duffy JE, *et al.* (2012) Climate Change Impacts on Marine Ecosystems. *Annual Review of Marine Science* **4**, 11-37.

Drummond A (2008) Reconstructing evolutionary bottleecks using the coalesecent.

Drummond AJ, Bouckaert RR (2015) *Bayesian evolutionary analysis with BEAST* Cambridge University Press, Cambridge.

Durban JW, Weller DW, Perryman WL (2017) Gray whale abundance estimates from shore-based counts off California in 2014/2015 and 2015/2016. . 4.pp.

Excoffier L, Foll M (2011) fastsimcoal: a continuous-time coalescent simulator of genomic diversity under arbitrarily complex evolutionary scenarios. *Bioinformatics* **27**, 1332-1334.

FitzSimmons NN, Limpus CJ, Norman JA, *et al.* (1997) Philopatry of male marine turtles inferred from mitochondrial DNA markers. *Proceedings of the National Academy of Sciences* **94**, 8912-8917.

Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**, 915-925.

Gailey G, Sychenko O, Tyurneva O, *et al.* (2020) Effects of sea ice on growth rates of an endangered population of gray whales. *Scientific Reports* **10**, 1-8.

Guindon S, Dufayard J-F, Lefort V, *et al.* (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**, 307-321.

Heller R, Brüniche-Olsen A, Siegismund HR (2012) Cape buffalo mitogenomics reveals a Holocene shift in the African human-megafauna dynamics. *Molecular Ecology* **21**, 3947-3959.

Heller R, Chikhi L, Siegismund HR (2013) The confounding effect of population structure on Bayesian skyline plot inferences of demographic history. *Plos One* **8**, e62992.

Heppell SS, Caswell H, Crowder LB (2000) Life histories and elasticity patterns: perturbation analysis for species with minimal demographic data. *Ecology* **81**, 654-665.

Ho SY, Lanfear R (2010) Improved characterisation of among-lineage rate variation in cetacean mitogenomes using codon-partitioned relaxed clocks. *Mitochondrial DNA* **21**, 138-146.

IUCN (2018) *The IUCN Red List of Threatened Species.* http://www.iucnredlist.org

Jackson JBC (2008) Ecological extinction and evolution in the brave new ocean. *Proceedings of the National Academy of Sciences* **105**, 11458-11465.

Kaschner K, Tittensor DP, Ready J, Gerrodette T, Worm B (2011) Current and Future Patterns of Global Marine Mammal Biodiversity. *Plos One* **6**, e19653.

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* **30**, 772-780.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* **34**, 772-773.

Lang AR, Weller DW, Leduc RG, Burdin AM, Brownell Jr RL (2010) Genetic differentiation between western and eastern (*Eschrichtius robustus*) gray whale populations using microsatellite markers. **139**, 1-18.

LeDuc RG, Weller DW, Hyde J, *et al.* (2002) Genetic differences between western and eastern gray whales (Eschrichtius robustus). *Journal of Cetacean Research and Management* **4**, 1-5.

Leigh JW, Bryant D (2015) popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6**, 1110-1116.

Leonard JA (2008) Ancient DNA applications for wildlife conservation. *Molecular Ecology* **17**, 4186-4196.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451-1452.

Mate BR, Ilyashenko VY, Bradford AL, *et al.* (2015) Critically endangered western gray whales migrate to the eastern North Pacific. *Biology Letters* **11**.

Mourier T, Ho SYW, Gilbert MTP, Willerslev E, Orlando L (2012) Statistical guidelines for detecting past population shifts using ancient DNA. *Molecular Biology and Evolution* **29**, 2241-2251.

Okonechnikov K, Golosova O, Fursov M, Team U (2012) Unipro UGENE: a unified bioinformatics toolkit. *Bioinformatics* **28**, 1166-1167.

Oksanen J, Blanchet FG, Kindt R, *et al.* (2010) Vegan: community ecology package. R package version 1.17-4. *http://cran. r-project. org>. Acesso em* **23**, 2010.

Paradis E (2010) pegas: an R package for population genetics with an integrated–modular approach. *Bioinformatics* **26**, 419-420.

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37.

Pérez-Alvarez MJ, Olavarría C, Moraga R, *et al.* (2016) Historical dimensions of population structure in a continuously distributed marine species: The case of the endemic Chilean dolphin. *Scientific Reports* **6**, 35507.

Phillips CD, Gelatt TS, Patton JC, Bickham JW (2011) Phylogeography of Steller sea lions: relationships among climate change, effective population size, and genetic diversity. *Journal of Mammalogy* **92**, 1091-1104.

Rambaut A (2012) FigTree v1. 4. *Molecular evolution, phylogenetics and epidemiology. Edinburgh, UK: University of Edinburgh, Institute of Evolutionary Biology*.

Rambaut A, Suchard M, Xie D, Drummond A (2016) Tracer v1.6.0, http://tree.bio.ed.ac.uk/software/tracer/.

Ramp C, Delarue J, Palsbøll PJ, Sears R, Hammond PS (2015) Adapting to a Warmer Ocean—Seasonal Shift of Baleen Whale Movements over Three Decades. *Plos One* **10**, e0121374.

Reeves RR, Ewins PJ, Agbayani S, *et al.* (2014) Distribution of endemic cetaceans in relation to hydrocarbon development and commercial shipping in a warming Arctic. *Marine Policy* **44**, 375-389.

Rice DWW, Rice AADW, Wolman AA (1971) *The life history and ecology of the gray whale (Eschrichtius robustus)* Stillwater, Okla. American Society of Mammalogists.

Roman J, Palumbi SR (2003) Whales Before Whaling in the North Atlantic. *Science* **301**, 508-510.

Shapiro B, Drummond AJ, Rambaut A, *et al.* (2004) Rise and fall of the Beringian steppe bison. *Science* **306**, 1561-1565.

Swartz SL, Taylor BL, Rugh DJ (2006) Gray whale Eschrichtius robustus population and stock identity. *Mammal Review* **36**, 66-84.

Taylor BL, Chivers SJ, Larese J, Perrin WF (2007) Generation length and percent mature estimates for IUCN assessments of cetaceans. *NOAA, NMFS, Southwest Fisheries Science Center Administrative Report LJ-07–01* **21**.

Wheeler DL, Church DM, Federhen S, *et al.* (2003) Database resources of the National Center for Biotechnology. *Nucleic Acids Research* **31**, 28-33.

Table 1. Genetic diversity statistics for eastern and western gray whales. Number of samples (*n*), observed number of haplotypes (*h*), haplotype diversity (h_d), number of segregating sites (*S*), nucleotide diversity (π), and average number of nucleotide differences between two sequences (*k*). Demographic change measured Fu and Li's coalescent-based estimators *D** and *F**

Population	***n***	***S***	***h***	h_d	π	***k***	***D****	***F****
Eastern	36	176	25	0.975	0.0018	30.1	0.00	-0.47
Western	38	120	9	0.723	0.0025	40.3	0.71	1.19
Combined	74	188	31	0.896	0.0023	37.4	-0.89	-0.69

None of the demographic tests were significant at $p = 0.05$.

$T_{\text{bottleneck}}$	300 years	16 generations
μ	4.0×10^{-8} bp^{-1} year^{1}	7.6×10^{-7} bp^{-1} gen^{-1}

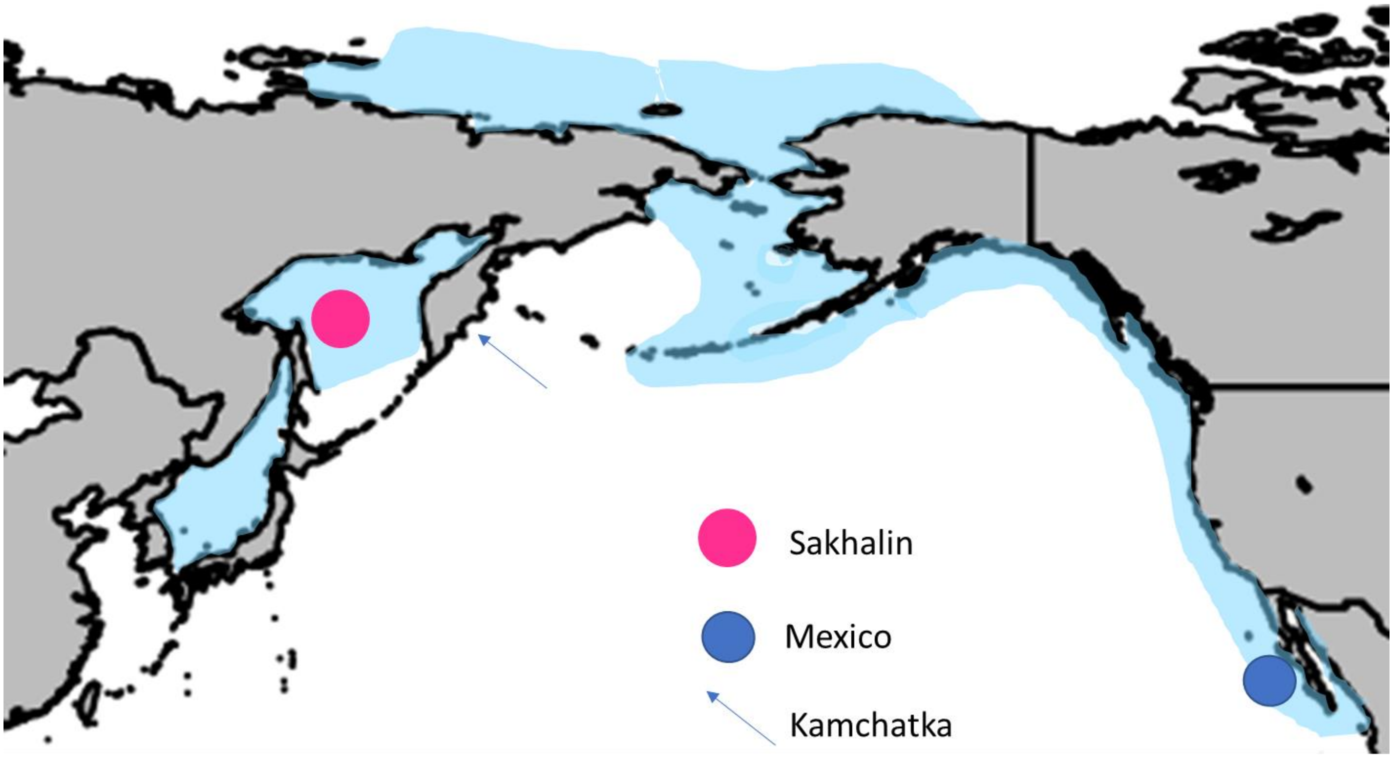

Figure 1. Figure 1.—Map of the North Pacific Ocean showing the range of gray whales (light blue) and the location of the Sakhalin (red) and Mexico (dark blue) sampling localities. The location of the Kamchatka population is denoted with an arrow.

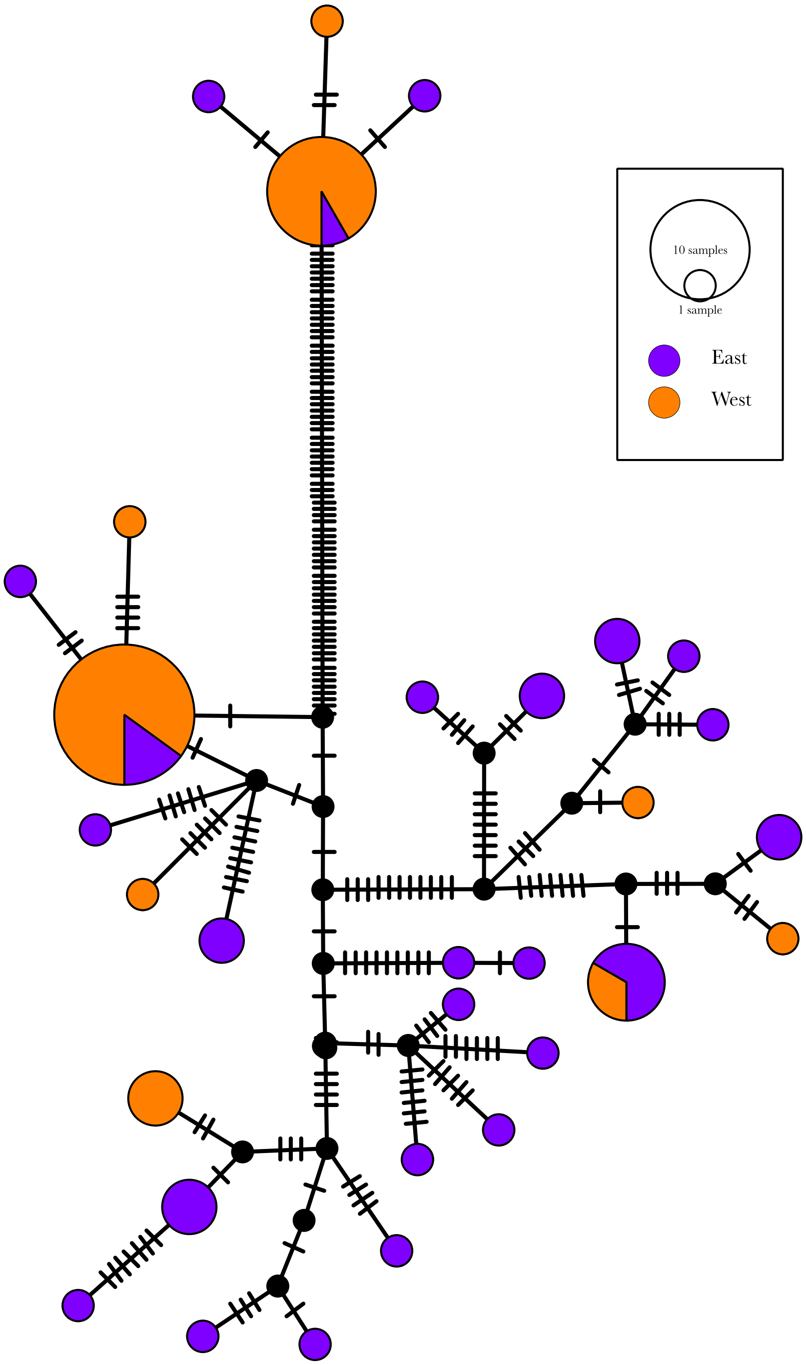

Figure 2. Haplotype network among the complete and the 16,414bp mitogenome. Breaks on the branches represent substitutions and the individual haplotype pie charts are scaled by the number of individuals with a given haplotypes. The top haplotype branch is separated by 79 substitutions.

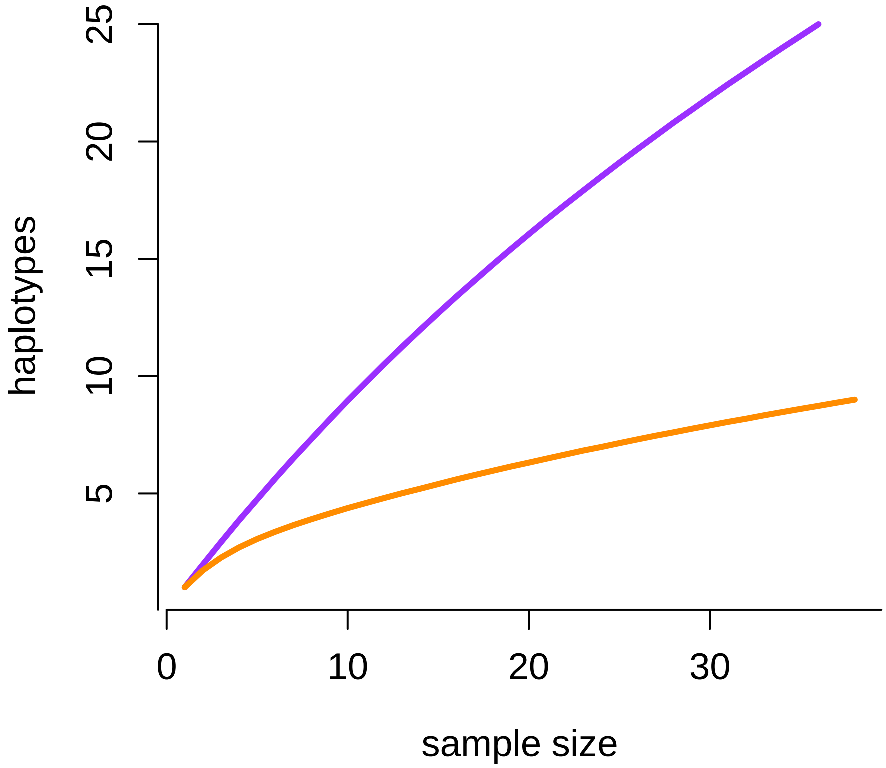

Figure 3. Rarefaction curves showing the number of haplotypes as a function of the number of sampled individuals for the eastern (purple) and western (orange) gray whale samples.

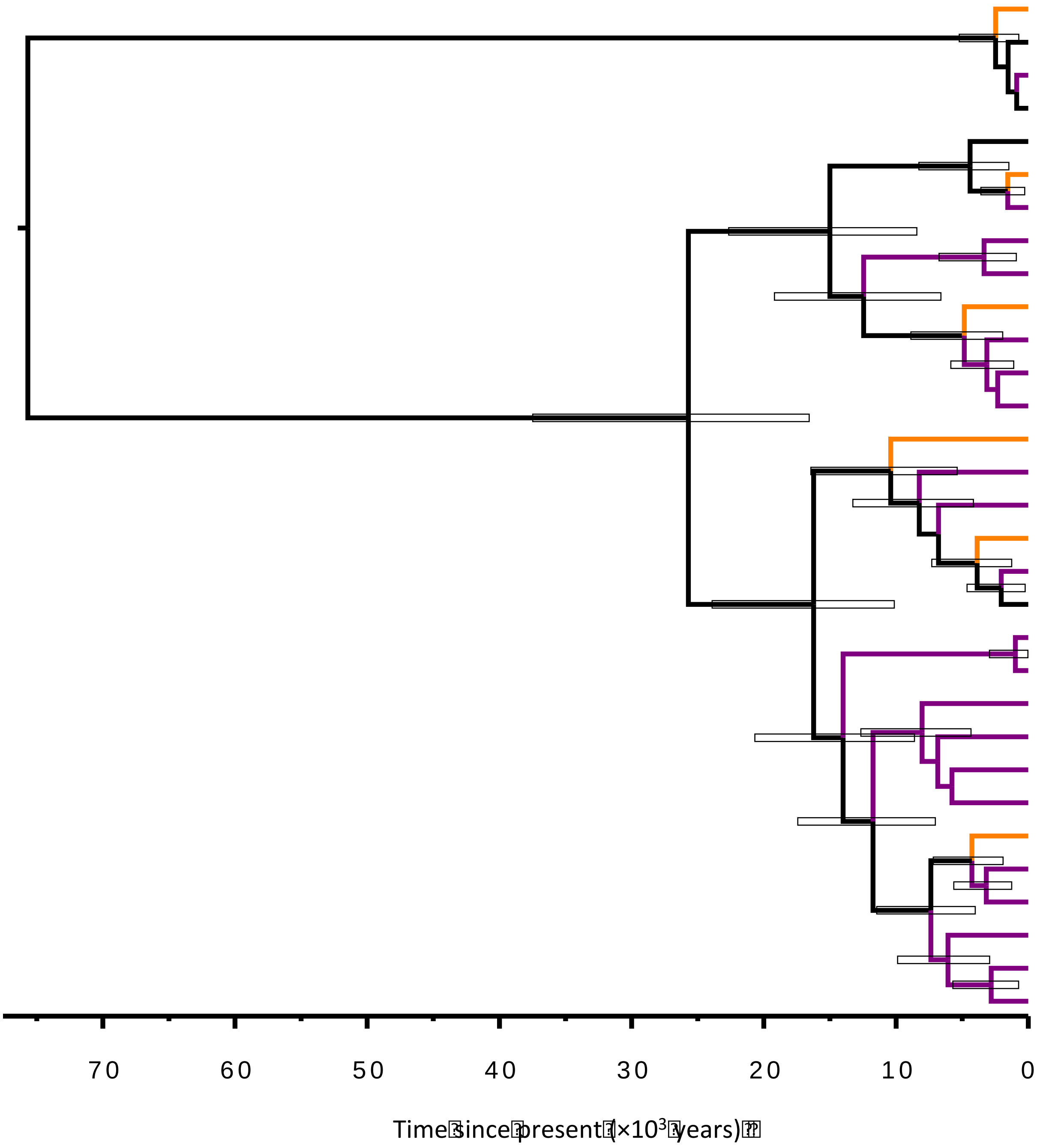

Figure 4. Time-calibrated phylogeny of the 31 unique mitogenome haplotypes. Lineage colors indicate whether a given haplotype is unique to the eastern (purple), western (orange) or shared between the two sampling locale (black). Scale bar represent 95% highest posterior density (HPD) for divergence estimates.

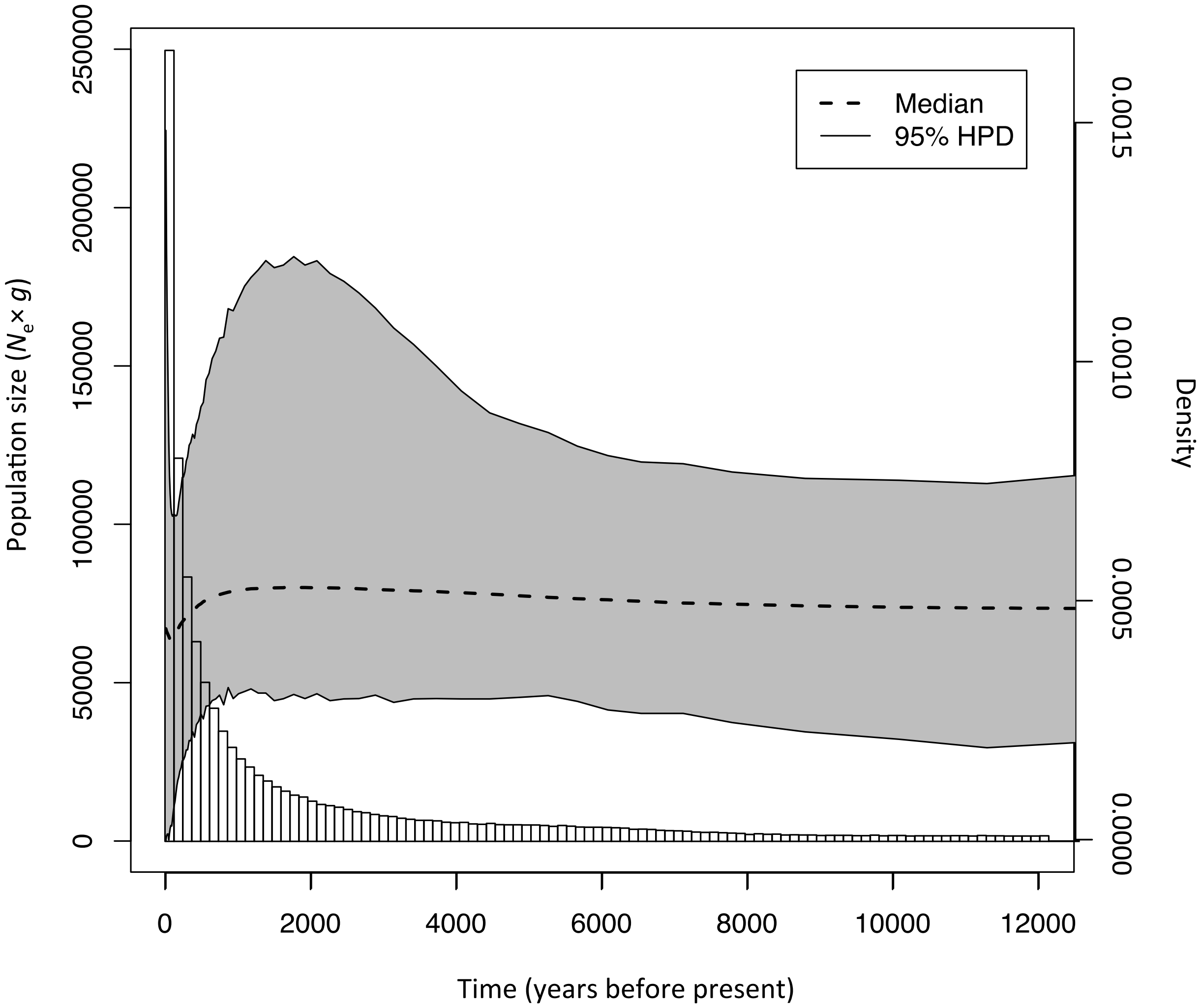

Figure 5. Extended Bayesian skyline plot for the gray whales. Plotted is the time scale in years before present. The dashed line represent the median population size and the gray area the 95% highest posterior density (HPD). Population size is given as the product of the female effective population size (N_e) and the generation time (g), the latter which for gray whales is 18.9 years. The histogram shows the density of tree events per time slice.