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Influence of Holocene habitat availability on Pacific gray whale (*Eschrichtius robustus*) population dynamics as inferred from whole mitochondrial genome sequences and environmental niche modeling

Anna Brüniche-Olsen,*, John W. Bickham, Celine A. Godard-Codding, Vladimir A. Brykov, Kenneth F. Kellner, Jorge Urban, and J. Andrew DeWoody

Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47905, USA (AB-O)
Department of Ecology and Conservation Biology, Texas A&M University, College Station, TX 77843, USA (JWB)
Institute of Environmental and Human Health, Texas Tech University (TTU) and TTU Health Sciences Center, Lubbock, TX 79409, USA (CAG-C)
National Scientific Center for Marine Biology, Russian Academy of Sciences, Far Eastern Branch, Vladivostok, Russia (VAB)
Departamento Academico de Ciencias Marinas y Costeras, Universidad Autonoma de Baja California Sur, Km 5.5 Carretera al Sur, Mezquitito, La Paz, BCS 23080, Mexico (JU)
Global Wildlife Conservation Center, State University of New York College of Environmental Science and Forestry, Syracuse, NY 13210, USA (KFK)
Department of Biological Sciences, Purdue University, West Lafayette, IN 47905, USA (JAD)

* *Correspondent: abruenic@purdue.edu*

Environmental changes since the Pleistocene and commercial whaling in the last few centuries have drastically reduced many whale populations, including gray whales in the North Pacific. Herein we use complete mitogenome sequences from 74 individuals to evaluate gray whale phylogeography and historical demography, then use environmental niche modeling to assess how habitat availability has changed through time for Pacific gray whales. We identify a large degree of haplotype sharing between gray whales sampled in Russian and Mexican waters, coupled with very limited matrilineal population structure. Confirming previous studies, our environmental niche models showed a decrease in available habitat during the Last Glacial Maximum, but we find no genetic signals of recent population declines in mitochondrial genomes despite both sustained habitat loss and a commercial whaling bottleneck. Our results illustrate the complex dynamics of baleen whale biogeography since the Holocene as well as the difficulty in detecting recent demographic bottlenecks from mitochondrial DNA sequences.

Key words: Cetacea, historical demography, mitogenome sequences, population structure, simulations

Anthropogenic change is rapidly altering marine ecosystems, with unknown consequences for the marine biota, including marine mammals (Jackson 2008; Doney et al. 2012). These human-induced changes are both direct (e.g., commercial whaling, ship strikes) and indirect (e.g., climate warming, pollution, industrial development). We are just beginning to understand how marine mammals responded to past environmental perturbations such as the Pleistocene glacial periods (Phillips et al. 2011). The genetic signatures of demographic events associated with such environmental change are important for conservation efforts because they inform predictions on how species may respond to ongoing and future environmental changes (Parmesan and Yohe 2003; Kaschner et al. 2011; Ramp et al. 2015). Many populations of baleen whales (Mysticeti) are particularly vulnerable after being decimated by unregulated commercial whaling in the 19th and 20th centuries, leading to very low population sizes (Roman and Palumbi 2003; Baker and Clapham 2004).

The gray whale (*Eschrichtius robustus*) is one species that already has been impacted both by anthropogenic and historical climate changes (Alter et al. 2015; Árnason et al. 2018; Brüniche-Olsen et al. 2018b). Since disappearing from the Atlantic Ocean due in large part to whaling, gray whales now are found only in the North Pacific Ocean (Alter et al. 2015),

where eastern and westerns assemblages are connected through limited gene flow (LeDuc et al. 2002; Lang et al. 2010; Alter et al. 2015; DeWoody et al. 2017; Brüniche-Olsen et al. 2018a). Gray whales in the eastern North Pacific number about 27,000 (Durban et al. 2015), and migrate along the coast of North America from winter breeding grounds in Mexico to summer feeding grounds primarily in the Bering Sea (Fig. 1). Thus, the migrations of gray whales (i.e., > 20,000 km annually—Mate et al. 2015) can rival or exceed those of any mammal. The western gray whale assemblage now numbers only about 200 individuals (Cooke et al. 2017). During the period of commercial whaling, the western gray whale migrated along the Asian coast from unknown winter breeding grounds, perhaps in the South China Sea, to summer feeding grounds in the Sea of Okhotsk. This population once was assumed to be extinct as a result of whaling but was rediscovered in the 1980s. Today, a small western population summers primarily off the northeastern coast of Sakhalin Island, Russia (Fig. 1). Uncertainty as to the geographic and genetic affiliation of the western gray whale was raised when satellite transmitters revealed that gray whales tagged near Sakhalin Island migrated to North American waters adjacent to Baja, Mexico, the known wintering grounds of the eastern gray whale (Lang et al. 2010; Mate et al. 2015).

Whether the current western population is a remnant of the historical western population hunted along the Asian coast, or if it represents a population recently founded by migrant eastern gray whales, or a mixed assemblage of the two, remains an open question. A study of nuclear single-nucleotide polymorphisms (SNPs) found mixed stocks on both sides of the Pacific (Brüniche-Olsen et al. 2018a), and a study of several mitochondrial DNA (mtDNA) genes of western whales found no evidence of unique haplotype lineages in whales with predominately western SNP genotypes (Brykov et al. 2019). Because of the absence of unique haplotype lineages, Brykov et al. (2019) concluded that the Sakhalin whales most likely are a recently isolated population founded by eastern gray whales. The fact that there are two common haplotypes found in all previous studies of the western population means that there could be unique haplotypes defined by mutations outside the surveyed regions. In this study, we revisit the question of the origin of this endangered population and the possible extinction

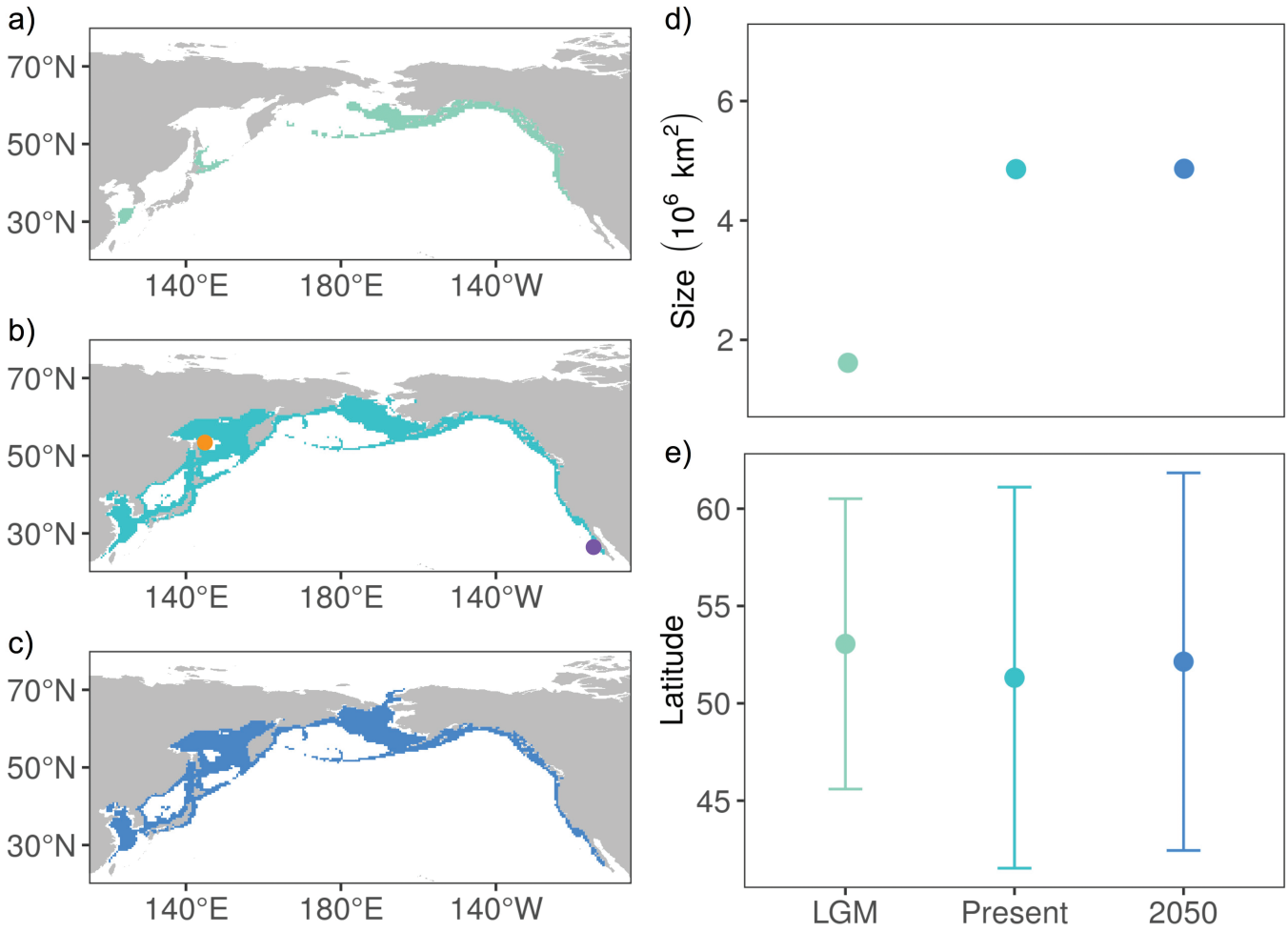

Fig. 1.—Habitat availability for Pacific gray whales during the Holocene. Environmental niche models representing areas with > 0.6 habitat suitability for (a) Last Glacial Maximum; (b) present day; and (c) year 2050. Sampling location for western gray whales (orange) of Sakhalin Island Russia, and eastern gray whales (purple) of Baja California, Mexico, are shown with dots on the present-day map in (b). Part (d) shows estimates of suitable habitat area (10^6 km^2), and (e) shows mean (± *SD*) latitude at each time period.

of the historical gray whale using the entire mtDNA genome sequence.

In addition, how gray whales will respond to anthropogenic climate warming also is a complex question. Environmental niche modeling of gray whale habitat for year 2100 suggests expanded suitable habitat (Brüniche-Olsen et al. 2018b) in part because decreased ice cover on their feeding grounds is correlated with a longer feeding season for reproductive females and higher seasonal calf survival (Gailey et al. 2020). However, the effects of climate warming on distribution of food resources, increased ship traffic in the Arctic, offshore oil drilling, and changes in commercial fishing practices, all could have detrimental impacts on gray whale survival (Coyle et al. 2007; Reeves et al. 2014).

The erosion of genetic diversity is a known factor that contributes to an increased probability of extinction. Conservation geneticists routinely use mtDNA sequences to test for genetic bottlenecks and to reconstruct deep historical population demography. However, with the advent of next generation sequencing, studies of demographic history using genotype-by-sequencing (GBS) and whole-genome resequencing methods are becoming more common (Shapiro et al. 2004; Heller et al. 2012; Carroll et al. 2019). The genetic detection of known demographic bottlenecks often is possible, but difficult, because it is influenced by a variety of factors including the pre-bottleneck level of genetic diversity, generation time, and migration rates (Busch et al. 2007; Taylor et al. 2007; Palsbøll et al. 2012). Nevertheless, pre- and post-whaling genetic evaluations of eastern gray whales have been used to assess the potential impacts of commercial whaling; the conflicting results illustrate the large uncertainty associated with inferring very recent demographic changes from DNA sequence data (i.e., on ecological timescales of decades to centuries as opposed to evolutionary timescales of millennia). For example, one recent study of eastern gray whales showed no change in genetic diversity for mtDNA sequences (~450 bp) or microsatellites (Béland et al. 2019). In contrast, other studies have recovered genetic signals of a recent bottleneck based on microsatellite data (Alter et al. 2009) with similar conclusions drawn from comparison of mtDNA sequences (383 bp) between modern and historical samples (Alter et al. 2012). Despite this disagreement among genetic studies, the recent census sizes of populations in the eastern North Pacific (N_c = 27,000) indicates they have been less impacted by commercial whaling than gray whales from the western North Pacific (N_c = 200–290—Cooke et al. 2017).

In this study, we use complete mitochondrial genomes from 74 gray whales sampled near Sakhalin, Russia, and Baja California, Mexico to: i) assess differentiation and gene flow among gray whale populations in the North Pacific Ocean; ii) reconstruct the female demographic history to recover past population dynamics; and iii) simulate different demographic scenarios to determine whether genetic signals of population decline could be detected with data sets similar to ours. In addition, we use environmental niche modeling to predict past and future changes in available gray whale habitat that might correlate with past demographic changes signaled by genetics, and to predict future population dynamics. These results will be useful for others who are considering the use of complete mitogenome sequences to infer demographic events in whales but also in other studies of recent population dynamics for large, long-lived species (e.g., elephants, giant tortoises, etc.).

Materials and Methods

Tissue collection and mitogenome sequencing.—We extracted DNA using the standard potassium acetate protocol (Sambrook and Russell David 1989) from tissue biopsy samples of 69 gray whales from the eastern and western North Pacific (Fig. 1; Supplementary Data SD1). The samples were collected between 2011 and 2016 using a 150-lb draw weight compound crossbow with 40 mm by 7 mm internal diameter tip arrows. The individuals used herein were previously genotyped at 91 autosomal SNP loci (Brüniche-Olsen et al. 2018a). DNA libraries were constructed using the TruSeq DNA Nano shotgun method using Unique Dual Indexed adapters from genomic DNA fragmented using a Covaris S3. Each sample was constructed into an isolated library, identifiable by its unique dual index. Samples were pooled and run on a single NovaSeq S6 run with 150-bp paired-end (PE) reads. We added PE reads from two gray whales sampled in the western North Pacific near Sakhalin Island, Russia (DeWoody et al. 2017) and three eastern gray whales sampled near Baja California, Mexico (DeWoody et al. 2017; Árnason et al. 2018), giving a total of 74 complete mitogenomes.

For mitogenome de novo assembly we used NOVOPLASTY v3.1 (Dierckxsens et al. 2016) using an eastern gray whale mitogenome (MF409244.1) as reference. To facilitate circular sequence alignment, we used MARS v1 (Ayad and Pissis 2017) and realigned the sequences using the neighbor-joining (NJ) method in MAFFT v6.903 (Katoh and Standley 2013). The sequence alignments were inspected visually in UGENE v1.32.0 (Okonechnikov et al. 2012). To quality check our data, we compared our de novo assemblies to data generated by mapping to our gray whale reference (MF409244.1) using BWA v0.7.17 (Li and Durbin 2009) and found them to be identical. We used ORFFINDER (Wheeler et al. 2003) with the mammalian mitochondrial genetic code to estimate the number of open reading frames (ORFs) in the main mitochondrial clades. To identify coding regions and tRNAs in the mitogenome, we used MITOS (Bernt et al. 2013) and GESEQ (Tillich et al. 2017) with the annotated gray whale mitochondrion (NC_005270) as reference. In light of recent work documenting interspecific hybridization and introgression among great whales (Árnason et al. 2018), we tested for the possibility of introgression by aligning gray whale mitogenomes to their close relatives, humpback whale (*Megaptera novaeangliae*, AP006467.1), fin whale (*Balaenoptera physalus*, KC572811.1), Antarctic minke whale (*Balaenoptera bonaerensis*, AP006466.1), blue whale (*Balaenoptera musculus*, MF409242.1), and sei whale (*Balaenoptera borealis*, AP0064470.1). The alignment and phylogeny were done with MAFFT, and the phylogeny visualized in FIGTREE v.1.4.2 (Rambaut 2012).

Genetic diversity and population structure.—We used DNAsp v5 (Librado and Rozas 2009) to calculate mtDNA 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 overall nucleotide diversity (π). Demographic changes were quantified with Fu's and Li's D^* and with Fu's F^* (Fu 1997). POPART (Leigh and Bryant 2015) was used to construct a median-joining haplotype network. We generated rarefaction curves using VEGAN (Oksanen et al. 2010) to estimate how much of the total haplotype diversity we sampled from each putative population. STRATAG (Archer et al. 2017) was used to perform a chi-square test to test for difference in haplotype frequencies (F_{ST}) between our samples from the eastern and western North Pacific (Wright 1951).

Intraspecific phylogeography.—A time-scaled mtDNA phylogeny for the gray whale haplotypes was constructed using maximum likelihood (ML) and Bayesian methods. A prior for the divergence time for the most divergent gray whale lineages was inferred using PHYML (Guindon et al. 2010). We identified lineages from time to the most recent common ancestor (TMRCA) by constructing a phylogeny of the unique gray whale haplotypes using fin whale (*B. physalus*, KC572811.1) and humpback whale (*M. novaeangliae*, AP006467.1) as outgroups. Based on the mtDNA phylogeny, we identified the main gray whale clades—in this case two—and selected a haplotype from each to represent the main gray whale clades of the phylogeny and used them for the TMRCA prior. A log-normal prior for TMRCA (mean = 80, SD = 0.15) was used based on TMRCA inferences from whole-genome sequences from eastern and western Pacific gray whales of 80 kya (60–100 kya 95% highest posterior density, HPD—Árnason et al. 2018) and from ancient mitochondrial data from the extinct Atlantic gray whale and the extant Pacific gray whale 79 kya (63–102 kya 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 based on the GESEQ results. The result was used to inform BEAST2 (Bouckaert et al. 2014), accounting for codon position rate variation (e.g., position 1, 2, and 3) in the coding regions (e.g., rRNA, tRNA, and CDS—Ho and Lanfear 2010; Supplementary Data SD2).

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

Historical demographic inference.—Demographic trajectories for the female effective population size (N_{ef}) were inferred using Bayesian coalescent samplers in BEAST2. Unequal sampling effort can confound demographic reconstruction (Heller et al. 2013). We therefore ran the demographic analysis twice, once for the entire data set (n = 74) and once for the data set divided into the two main clades A (n = 15) and B (n = 59; see the phylogeny in Fig. 3). We used a log-normal clock and our estimated substitution rate of 4.0×10^{-8} bp^{-1} year^{-1} (2.3×10^{-8} to 6.0×10^{-8} 95% HPD). To quantify changes in $N_{ef,}$ we used the "extended Bayesian skyline plot" (EBSP) because we were interested in the "sum(indicators.alltrees)" parameter, which describes the number of likely population size changes in the data. Multiple runs were undertaken for each model and checked for convergence in TRACER. To convert EBSP's composite population size parameter ($N_{ef} \times g$) to female effective population size (N_{ef}), we used a generation time (g) of 18.9 years as the midpoint between 15.5 and 22.3 years (Rice et al. 1971; Heppell et al. 2000, respectively). This estimate of generation time was chosen to facilitate comparison to demographic parameters from whole-genome data (Brüniche-Olsen et al. 2018b).

Simulating demographic scenarios.—We carried out a posteriori simulations to determine if our whole mitogenome sequences provided meaningful biological insights into the identification of recent demographic events. Using FASTSIMCOAL2 (Excoffier and Foll 2011), we simulated demographic scenarios for the putative population dynamics of gray whales and used the simulated data sets in a BEAST2 EBSP analysis to investigate whether we recovered trajectories similar to those generated from our empirical mitogenomic data. We simulated three scenarios: a simple population size reduction, a severe bottleneck followed by recovery, and a weak bottleneck followed by recovery. For each scenario, we simulated 74 sequences of 16,414 bp in length. We assumed that Pacific gray whales comprised a single demographic population with random mating and thus did not include migration in the model. We simulated one locus and one recombination block using a mutation rate $\mu = 4.0 \times 10^{-8}$ bp^{-1} year^{-1} corresponding to 7.6×10^{-7} bp^{-1} gen^{-1}. The transition/transversion rate was set to 0.66 and, based on our empirical data set, the HKY+G+I substitution model was identified as the best fit with BMODELTEST (Bouckaert and Drummond 2017). We generated ten random parameter sets from the priors.

We set the current population size to 21,000 – 23,000 N_c (Durban et al. 2017), and the past population size to 95,000 – 97,000 N_c (Alter et al. 2012). The bottleneck population size prior was severe (900 – 1,000 N_c), weak (9,000 – 10,000 N_c), or a population reduction to contemporary N_c (21,000 – 23,000 N_c—Alter et al. 2012). We used a prior for the timing of the bottleneck assuming the whaling started 9 – 10 generations ago in the mid-19th century and lasted until the end of the 20th century, some 2 – 3 generations ago (Sumich 2014). We also simulated a more ancient bottleneck starting 22 – 23 generations ago and lasting until 6 – 7 generations ago, to investigate if the success in detecting population size changes would be time dependent (Palsbøll et al. 2012). We converted between census population size (N_c) and N_{ef} such that $N_c = 6N_{ef}$, assuming that i) the

number of breeding males is equal to the number of breeding females ($N_e = 2N_{ef}$); ii) the total adult population size (N_T) is twice as large as the effective population size ($N_T = 2N_e$); and iii) that juveniles represent 1/3 of the census population size ($N_c = 1.5N_T$) (Roman and Palumbi 2003; Alter et al. 2007; Alter et al. 2012).

Environmental niche modeling.—We quantified suitable habitat for gray whales with an ecological niche model (ENM) built using AQUAMAPS (Ready et al. 2010; Kaschner et al. 2011). We quantified habitat for three time periods: the Last Glacial Maximum (LGM; 26.5 – 19 ka ago), present (year 2020), and future (year 2050). We used information from GLAMAP to reconstruct LGM habitat (Vogelsang et al. 2001; Schäfer-Neth and Paul 2003). The ENM was parameterized based on depth, temperature, salinity, and sea ice concentration envelopes (Supplementary Data SD3; Alter et al. 2015). For the LGM map, mean annual sea ice concentration was approximated as the mean proportion of time a given cell was covered by ice based on the GLAMAP data (Alter et al. 2015). We defined suitable habitat as probability > 0.6 based on the model, which often is used as cutoff for suitable habitat for marine mammals (Kaschner et al. 2011; Louis et al. 2020). Our analysis is similar to previous studies of ENM for gray whales (Alter et al. 2015; Brüniche-Olsen et al. 2018b) but differs by: i) including a threshold cutoff for suitable habitat; ii) our inclusion of LGM climate ENM in the current study; and iii) extending the climate ENM to the year 2050.

Results

Genetic diversity and population structure.—We evaluated mitogenome sequences from 74 gray whales, including 36 from Mexico and 38 from Sakhalin (Table 1). The mean depth of mtDNA sequence coverage was 250× per individual, providing a high degree of certainty for haplotype identification. We found no evidence for introgressive hybridization among gray whales and related baleen whales (Supplementary Data SD5). When analyzed independently, we saw no evidence of demographic changes based on D^* and F^* in either population. The eastern samples had a higher number of haplotypes and higher haplotype diversity ($h = 25$ and $h_d = 0.975$) compared to the western samples ($h = 9$ and $h_d = 0.723$; Table 1), concordant with the much larger contemporary census size of the eastern gray whale population. Despite their reduced number of haplotypes (only about 1/3 as many as the eastern gray whales), the western samples had higher π and k, indicating that haplotypes were (on average) more divergent from one another. We identified similar levels of control region diversity found in previous studies (Alter et al. 2015; Supplementary Data SD1 and SD5).

Our haplotype network identified two main lineages that were separated by 79 substitutions (Fig. 2). A similar deep branching structure has been uncovered in previous analyses of gray whale mtDNA including noncoding control region sequences (Alter et al. 2015) as well as the control region plus multiple short coding regions (Meschersky et al. 2015; Brykov et al. 2019). We considered that one of the lineages might represent a nuclear copy of a mitochondrial pseudogene

Table 1.—Genetic diversity statistics for eastern and western Pacific gray whales. Number of samples (n), number of segregating sites (S), observed number of haplotypes (h), haplotype diversity (h_d), nucleotide diversity (π), and average number of nucleotide differences between two sequences (k). Demographic change measured Fu's and Li's coalescent-based estimators D^* and F^*. None of the demographic tests (D^* and F^*) were significantly different from zero at $P = 0.05$.

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

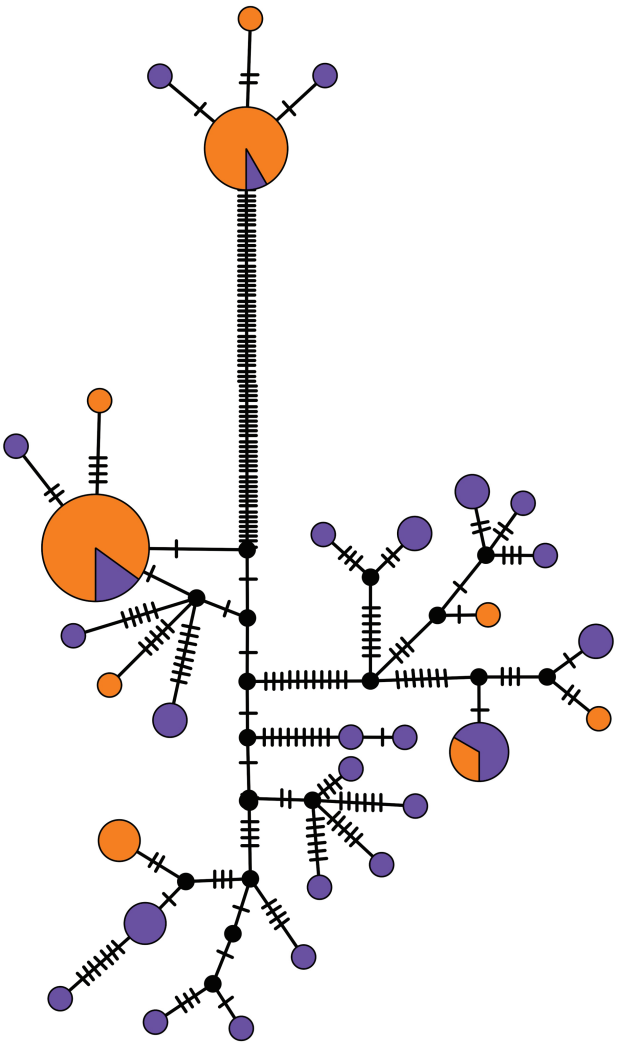

Fig. 2.—Haplotype network for Pacific gray whales among the complete 16,414-bp mitogenome. Breaks on the branches represent substitutions; the individual haplotype pie charts are scaled by the number of individuals with a given haplotype (i.e., circle size corresponds to haplotype frequency). The branch lengths are not proportional to genetic distance. The top haplotype branch is separated by 79 substitutions. Whereas eastern (purple) gray whales possess numerous private haplotypes, few are observed for western (orange) gray whales.

(numts—Triant and DeWoody 2007), but both appeared to be functional molecules that consisted of the same ORFs. There was no pronounced phylogeographic structure in the haplotype network. Eastern gray whales were found throughout the haplotype network and mainly represented by singleton haplotypes, whereas the diversity was dominated by two common haplotypes in the western samples (Supplementary Data SD6). A chi-square test showed that there was significant difference in haplotype frequencies (F_{ST} = 0.024) between our samples from the eastern and western North Pacific (χ^2 = 54.8, *d.f.* = 30, P = 0.004), but with only two geographic sampling sites, we could not test whether this differentiation could be ascribed to the null hypothesis of isolation-by-distance. Rarefaction curves of sampling effort (Supplementary Data SD7) 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.

Intraspecific phylogeography.—Our phylogenetic analysis showed that an uncorrelated log-normal clock fits our data set better than a strict clock model ("uclStdev" = 0.17). The phylogeny is based on estimates of TMRCA inferred from whole-genome sequences (Árnason et al. 2018) and from the control regions of the mtDNA (Alter et al. 2015); the previously reported TMRCA estimates were marginally more recent than our estimate of ~76.5 kya (55.0 – 99.5% HPD; Fig. 3). Most branches coalesced 20 – 23 kya, during the LGM, likely reflecting the LGM bottleneck (Brüniche-Olsen et al. 2018b). Private haplotypes were observed in both eastern and western Pacific sampling sites (Table 1; Fig. 2), but private clades were found in the eastern population only. These were recent (< 15 kya) suggesting either i) recent but limited maternal gene flow from the eastern to the western population; ii) loss of lineages in the western gray whale due to small population size causing rarer haplotypes to be lost more frequently; iii) sampling error due to the small number of eastern gray whales sampled compared to the population size; or iv) incomplete lineage sorting.

Historical demographic inference.—Our EBSP analysis of the entire data set revealed the number of demographic changes (described by the "sum(indicators.alltrees)" parameter) to have a median = 1 [0 – 3 95% HPD] but we could not reject that a null model of constant population size was most likely because the 95% HPD included zero. The demographic trajectory showed that the long-term population size was ~80,000 ($N_{ef} \times g$) corresponding to a median N_{ef} ~ 4,200 and N_c ~ 25,200 assuming a generation time of 18.9 years (Fig. 4a). A slight recent decline in population size was observed ~70,000 ($N_{ef} \times g$) corresponding to a median N_{ef} ~ 3,700 and N_c ~ 22,200. There were large uncertainties associated with the $N_{ef} \times g$ estimates, in particular recent (< 5,000 years ago) $N_{ef} \times g$ (Fig. 4a confidence interval [CI]: 0 – 200 k). Most of the demographic signal occurred very recently (< 500 years ago), suggesting that we quickly lose resolution as we go back in time with the mitogenome data set (Supplementary Data SD8).

When we accounted for population structure (i.e., the deep split between the two main clades) by inferring demographic trajectories for the two main clades separately, we found that clade A (n = 15) did not have sufficient sequence variation to yield ESS > 200; the data therefore are not presented. Clade B yielded ESS > 200 and had a median of two demographic changes, showing an increase in N_{ef} after the LGM followed by a more recent decline (Fig. 4b). However, we still could not reject a constant population size because the 95% HPD included zero [0 – 8 95% HPD]. The post-LGM population size was ~25,000 ($N_{ef} \times g$), corresponding to a median N_{ef} ~ 1,300 and N_c ~ 7,800 and reached a peak population size 2,000 years ago of ~120,000 ($N_{ef} \times g$) corresponding to N_{ef} ~ 6,300 and N_c ~ 37,800. The current population size is similar to the inferred post-LGM population size (Fig. 4b).

Simulating demographic scenarios.—The simulated demographic scenarios showed variation both in performance (the number of simulated data sets that produced EBSP with prior and posterior ESS > 200), and sensitivity in detecting a population size change (Fig. 5a). None of the simulations for a recent severe population bottleneck had ESS >200 and therefore are not included in Fig. 5b. The percentage of simulations that either could not reject a constant population size (95% HPD = [0, n]) and that rejected a constant population size (95% HPD = [1, n]) varied considerably among the scenarios. None of the recent population demographic scenarios could exclude a constant population size of gray whales since the LGM. The ancient population change scenarios had better detection rates as bottleneck intensity increased with weak bottleneck with recovery (17%) and severe bottleneck with recovery (37%). Overall, the scenario of an ancient severe bottleneck followed by recovery was the most likely to be recovered with mitogenome data, but even those severe scenarios were detected < 50% of the time (Fig. 5c).

Environmental niche modeling.—Our environmental niche modeling showed that the extent of suitable habitat for gray whales has increased ~300% from the LGM (1.62×10^6 km^2) to present (4.86×10^6 km^2) and is expected to remain steady or increase very modestly through 2050 (4.87×10^6 km^2; Fig. 1). We did not observe a shift in mean latitude of suitable habitat, but our modeling showed that the extent of suitable habitat increased after the LGM due to latitudinal expansions and that the available habitat is expected to further increase northward with Arctic warming (Moore and Huntington 2008).

Discussion

During glacial periods, many species of great whales experienced a drastic decline in population size due to reduction in suitable habitat, whereas interglacial periods led to population expansions associated with habitat expansion due to warmer climate (Árnason et al. 2018). Most recently, the LGM caused drastic population size reductions of some species that were exacerbated by recent commercial whaling (Roman and Palumbi 2003; Baker and Clapham 2004). These sequential population reductions are a concern as genetic diversity is lost during periods of small population size, which could lead to population extirpation in extreme cases (Leroy

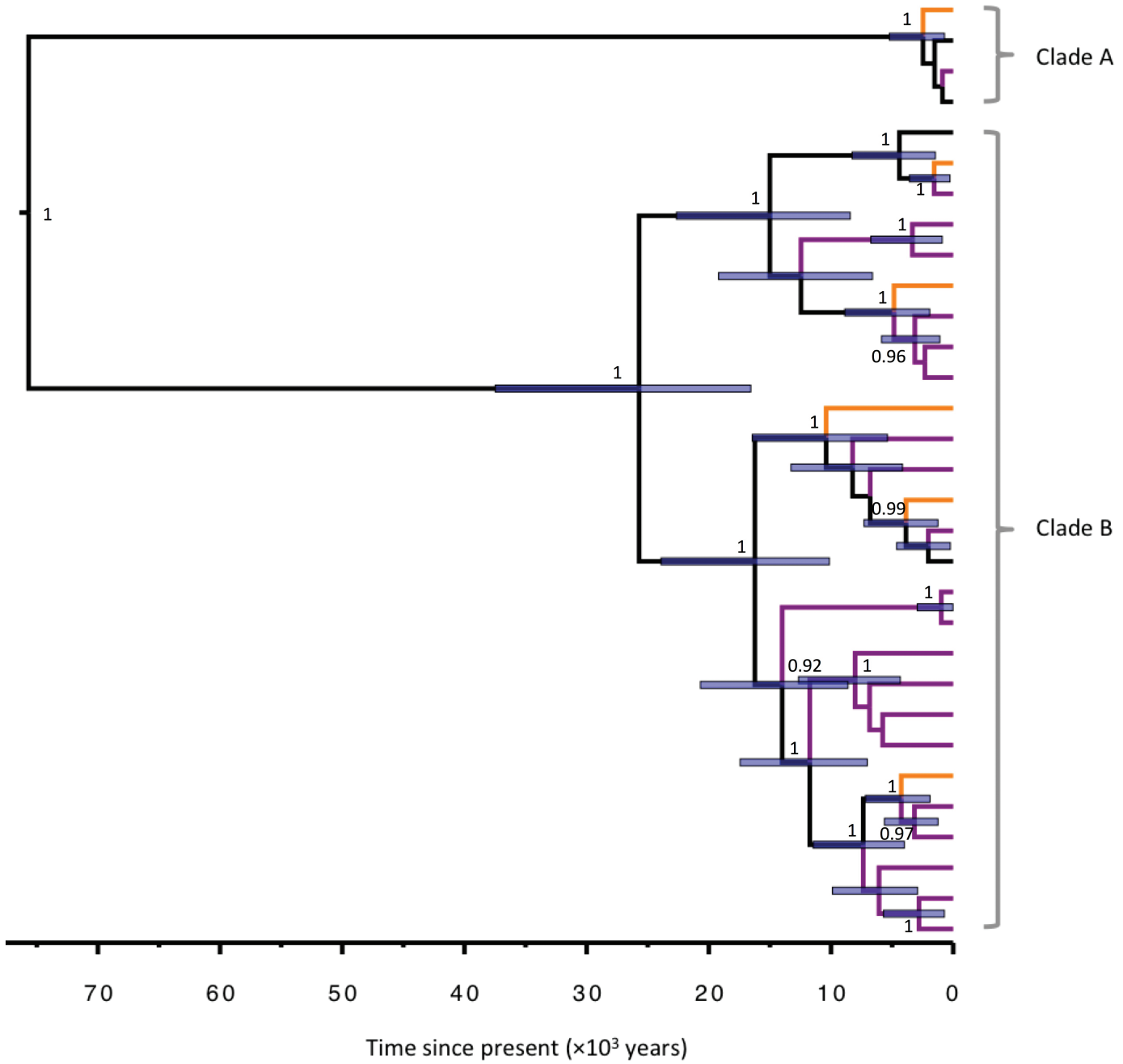

Fig. 3.—Time-calibrated phylogeny of the 31 unique mitogenome haplotypes from Pacific gray whales. Lineage colors indicate whether a given haplotype is unique to the eastern (purple), western (orange), or shared between the two sampling localities (black). Scale bar represent 95% highest posterior density (HPD) for divergence estimates. Nodes with > 0.7 posterior are given. The two main clades in the phylogeny are indicated as clade A and B.

et al. 2018). Gray whales are thought to have experienced substantial declines in the Pleistocene (Brüniche-Olsen et al. 2018b) and Holocene (Alter et al. 2007, 2012, 2015). Herein we examine mtDNA population dynamics of North Pacific gray whales, identifying limited maternal phylogeographic structure, higher genetic diversity in the eastern gray whale population, and reduction in available habitat during the Holocene.

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 (LeDuc et al. 2002; Lang et al. 2010; Brüniche-Olsen et al. 2018a; Brykov et al. 2019). Nuclear SNP markers established that both the eastern and western gray whale populations are mixed-stock assemblages, with one genetic background predominating in whales sampled from the Mexican wintering grounds and another background predominating in whales sampled at summer feeding grounds off the coast of Sakhalin Island (Brüniche-Olsen et al. 2018a).

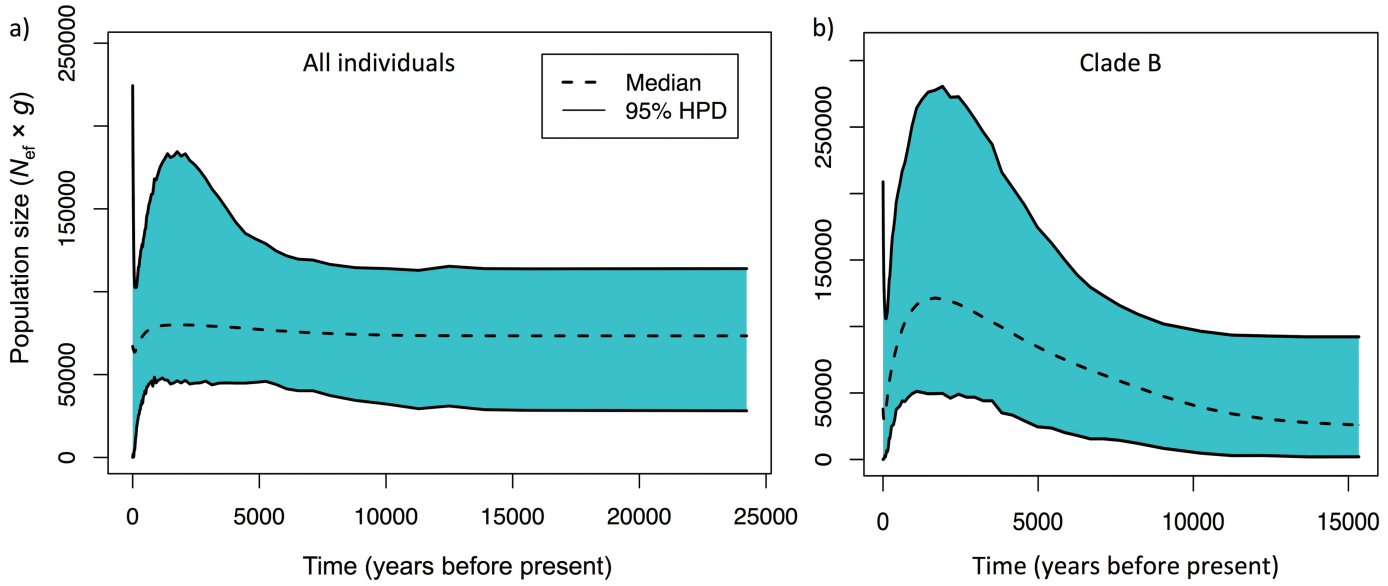

Fig. 4.—Extended Bayesian skyline plot (EBSP) for the Pacific gray whales. EBSPs are shown for (a) the entire data set; and (b) accounting for population structure for clade B (see Fig. 3). The dashed line represents the median population size and the gray area the 95% highest posterior density (HPD). Population size is given as the product of the female effective population size (N_{ef}) and the generation time (g), the latter of which for gray whales is estimated at 18.9 years, as described in the text. As the 95% HPD includes zero in both cases, a constant population size cannot be rejected.

We know from photo identification (photo ID) and genetics that male and female gray whales of both genetic backgrounds migrate from Sakhalin to Mexico (Weller et al. 2012; Brykov et al. 2019), and we know from telemetry data that gray whales migrate between Sakhalin and Mexico (Mate et al. 2015). Our complete mitogenome data confirm and extend previous studies (LeDuc et al. 2002; Lang et al. 2010; Brykov et al. 2019). Despite the significant difference in haplotype frequencies between the eastern and western populations, the mtDNA haplotype networks provide no evidence of unique lineages within the western gray whale population (Fig. 2), as might be expected if females were strongly philopatric due to natal homing or social facilitation and extended temporal isolation on opposite sides of the North Pacific Ocean basin (FitzSimmons et al. 1997).

Population dynamics and sensitivity of coalescence analyses.—Studies of mtDNA control region sequences from (historical) Atlantic and Pacific gray whales show that there was gene flow not only 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 than by sampling additional whales from Sakhalin (Supplementary Data SD7). This mirrors the IUCN status, with the eastern gray whale being considered of least conservation concern, whereas the western gray whale is considered endangered (Cooke et al. 2017; Cooke et al. 2018). The IUCN does not explicitly consider genetic data as part of its listing framework—also not explicitly excluding it—but our view is that the mtDNA data herein certainly do not reflect a geographically structured split in the gray whale gene pool as might be expected if the two populations were genetically and demographically independent over evolutionary time. The mtDNA genome represents only a single locus, however, and extensive nuclear data (e.g., GBS, whole-genome resequencing) are needed to better resolve population structure and demographic history in gray whales.

With respect to the two divergent mtDNA lineages encountered in this (and other) work (Figs. 2 and 3), we evaluated several possibilities. First, we rejected the idea that one lineage represents a numt given that both eastern and western lineages contain the same ORFs. The different genetic codes and the disparate evolutionary rates between nuclear and mitochondrial genomes almost certainly would have resulted in disparate ORFs if one of these lineages was a numt (Triant and DeWoody 2007). We next considered the possibility of interspecific hybridization, introgression, and ultimately mitochondrial capture. Great whale introgression has been recently revealed by virtue of whole-genome sequences (Árnason et al. 2018), but our phylogenetic analyses are inconsistent with an mtDNA capture scenario for the mitogenome (Supplementary Data SD4). Finally, we were left to consider demographic effects that may have exacerbated genetic drift.

Our molecular estimate of the current population size, N_c ~ 22,200 – 25,200, fits with the visually estimated census size N_c ~ 27,000 (Durban et al. 2017). A slight recent decline in population size was observed (Fig. 4a), which may be due to a scattering phase (e.g., recent coalescent events resulting from within or between deme genetic variation) between the two main clades (Wakeley 1999; Pannell 2003; Heller et al. 2013). The deep divergence ~75 kya between major clades

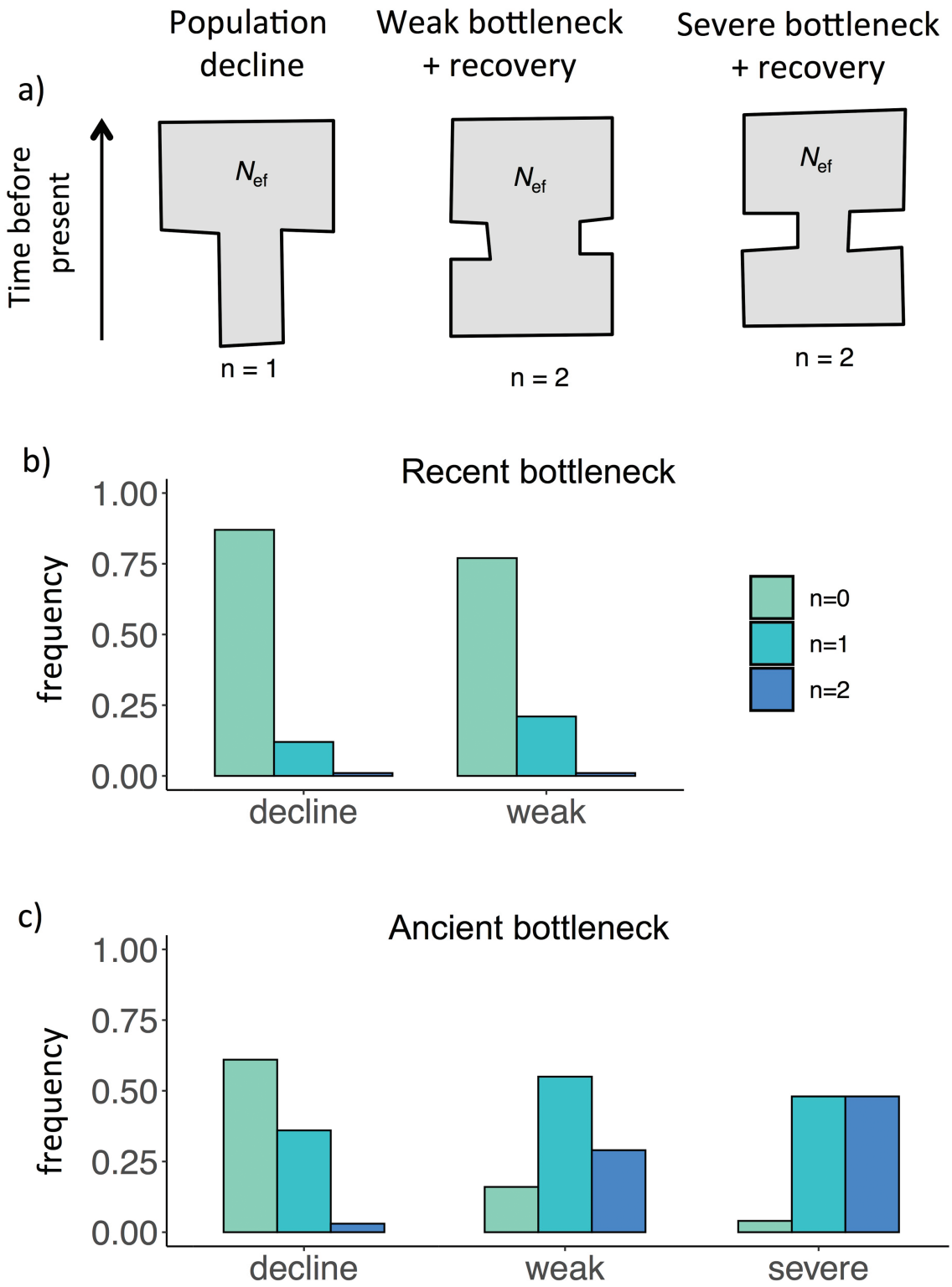

Fig. 5.—Success of detecting population size changes in simulated demographic scenarios. (a) shows the three scenarios (not drawn to scale) and the simulated number of changes (n = 1 or 2); (b) and (c) show the frequency of median number of population size changes (n = 0, 1, or 2) detected for each scenario based on (b) recent (9 – 10 to 2 – 3 generations ago) and (c) ancient (22 – 23 to 9 – 10 generation ago) bottlenecks. The frequency for detecting the correct number of simulated population size changes was low for all the ancient scenarios, and none of the recent scenarios could reject a constant population size.

(Fig. 3) predates the LGM, and most of the branches coalesced 20 – 23 kya ago, potentially reflecting the LGM bottleneck (Brüniche-Olsen et al. 2018b). We used a divergence date prior probability based on whole-genome sequences (~80 kya—Árnason et al. 2018) and control region mtDNA (~79 kya—Alter et al. 2015). TMRCA for the Pacific gray

whales might be more recent—as suggested by Alter et al. (2015)—but even with a divergence time of ~45 kya, most of the coalescent events still would have occurred prior (> 5 kya) to the onset of commercial whaling. Thus, the mitochondrial diversity in gray whales was partially depleted prior to commercial whaling. This is undoubtedly due at least in part to the fact that gray whale population sizes fluctuate with climatic conditions. For example, dispersal between the Atlantic and Pacific was limited during the LGM (Alter et al. 2015) and historical population declines have been associated with glacial periods (Pyenson and Lindberg 2011; Brüniche-Olsen et al. 2018b). Furthermore, feeding habitat in the Arctic has shifted latitudinally as a result of climate change (Alter et al. 2007; Pyenson and Lindberg 2011) with expanded Arctic habitat during interglacial periods leading to increased population sizes (Brüniche-Olsen et al. 2018b).

Using whole mitogenome sequences, we failed to detect the recent commercial whaling bottleneck both for the entire data set (Fig. 4a) and when accounting for population structure (Fig. 4b). Unfortunately, our simulations illustrate that contemporary gray whale mitogenomes provide insufficient resolution to detect very recent demographic bottlenecks (i.e., those that have occurred in the last dozen or so generations) using the techniques described herein (Fig. 5). Many studies have used mtDNA to reconstruct demographic history (e.g., Shapiro et al. 2004; Heller et al. 2012), but for long-lived species, the resolution for such analyses is suboptimal if the bottleneck was subtle or if it was followed by rapid demographic recovery (Mourier et al. 2012). This situation is exacerbated when substitution rates are slow (e.g., due to long generation times and/or slow mutation rates due to metabolic or other constraints—Martin and Palumbi 1993).

The power of EBSP to detect multiple population size changes with a single locus is limited. Our results show that using only the mitogenome makes any change in population dynamics extremely difficult to detect (Fig. 5) if the change is recent or subtle. Incorporating nuclear markers along with mtDNA can help resolve some of the signals related to gene flow (Carroll et al. 2019). To capture very recent changes such as the ones related to commercial whaling, temporal sampling (e.g., pre- and post-event) is most desirable (Díez-del-Molino et al. 2017), as has been done for eastern gray whales (Alter et al. 2012), North Atlantic right whales (*Eubalaena glacialis*—Rosenbaum et al. 2000), humpback whales (*M. novaeangliae*—Béland et al. 2019), and bowhead whales (*Balaena mysticetus*—Foote et al. 2013). Whole-genome sequence data has proven a better tool for detecting recent demographic changes due to the much higher resolution that the abundance of markers provide, both when using contemporary (Abascal et al. 2016; Ceballos et al. 2018) or temporal sampling (van der Valk et al. 2019).

Gray whale habitat during the Holocene.—During the LGM, many cetaceans experienced population declines due to climate-mediated reduction in habitat (Morin et al. 2015; Brüniche-Olsen et al. 2018b). By the year 2050, a global shift in cetacean species richness is expected to occur. Higher latitudes (above 40°) are expected to experience an increase in species richness at both the Southern and Northern hemisphere due to ocean warming, while lower latitudes are expected to experience a decline in cetaceans (Kaschner et al. 2011). Our ENM shows that suitable gray whale habitat in the Pacific has increased by nearly 300% since the Holocene (Fig. 1); we did not model habitat in the Atlantic but see Alter et al. (2015). The extent of suitable habitat in the Pacific was substantially reduced during the LGM compared to present-day and near-future projections. Although we would expect the suitable habitat to shift to lower latitudes during the LGM (Fig. 1a), as Arctic sea ice extended further south compared to its current distribution (Fig. 1b), the mean latitude during the Holocene remained constant (Fig. 1d). This likely reflects that habitat expansion not only occurs latitudinally but also longitudinally. Future climate warming should lead to stability or even modest increases in available habitat for gray whales in the Pacific (Fig. 1c; Moore and Huntington 2008), whereas other cetaceans, like the ice-dependent narwhals (Louis et al. 2020) and bowhead whales (Foote et al. 2013), are likely to experience a reduction in available habitat. These short-term (~30 year) projections illustrate the diverse responses of cetaceans to short-term global warming (Kaschner et al. 2011). Developing more nuanced and longer-term (> 30 year) climate models may help shed light on how the cetacean community will adapt to future climate change; however, those efforts are beyond the scope of this paper.

Here we present a large data set of complete mitogenomes from 74 gray whales, and environmental niche modeling of gray whale habitat availability during the Holocene. Our study has produced 69 new mitogenomes from eastern and western gray whales. Based on integrative analyses of genomic and environmental data, which data can have the potential to provide key insights into the biology of whales and other mammals. Our mtDNA data show notably higher genetic diversity in the larger contemporary eastern gray whale population relative to whales from the western North Pacific, consistent with their census population size estimates and previous mtDNA studies. We found limited maternal population structure in North Pacific gray whales and some evidence for matrilineal gene flow from the east to the west. Our ENM indicates that available gray whale habitat was reduced during the LGM relative to modern day, and that reduction in LGM habitat likely resulted in a long-term demographic decline that depleted haplotype diversity and, perhaps, obscured genetic signatures of the recent bottleneck known to be associated with commercial whaling.

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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 Subsecretaría de Gestión Para La Protectión Ambiental of the Mexican Dirección 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.

Conflict Of Interest

The authors declare no conflict of interest.

Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Overview of Pacific gray whale tissue sample ID, sampling year, sampling location, and sex as inferred in Brüniche-Olsen et al. (2018a).

Supplementary Data SD2.—Gray whale mitogenome data blocks as defined by PARTITIONFINDER based on annotation results from GESEQ and MITOS. For each region ID a name, annotation, and the number of base pairs (bp) it covers are given.

Supplementary Data SD3.—Environmental variables used in AQUAMAPS to generate maps of suitable habitat for gray whales during the Holocene. Values are from Alter et al. (2015).

Supplementary Data SD4.—Cladogram of how the gray whale mitogenomes cluster compared to other great whales. Note the two gray whale sequences are monophyletic with respect to each other, indicating that neither are derived from another species via introgression followed by mitochondrial capture. Made with MAFFT and visualized in FIGTREE. The labels refer to NCBI accession numbers.

Supplementary Data SD5.—Genetic diversity statistics for Pacific and Atlantic gray whales based on 463-bp mtDNA control region. Number of samples (n), observed number of haplotypes (h), haplotype diverstiy (h_d), number of segregating sites (S), nucleotide diversity (π), and average number of nucleotide differences between two sequences (k). Demographic change measured Fu's and Li's coalescent-based estimators D^* and F^*. Samples labeled with # are data from Alter et al. (2015)

Supplementary Data SD6.—Haplotype network for 284 Pacific and Atlantic gray whales based on 463-bp mtDNA control region sequence. Haplotypes are color-coded according to whether they are the western (purple) and eastern (orange) gray whales from the current study or data from Alter et al. (2015) representing eastern (yellow), western (pink), and Atlantic (blue) gray whales.

Supplementary Data SD7.—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.

Supplementary Data SD8.—Density of demographic events in the extended Bayesian skyline plot (EBSP) analysis. The majority of the demographic events occur very recently (< 500 years ago), suggesting that, with the mitogenome data set, we quickly lose resolution as we go back in time.

Data Availability

GenBank accession codes for the 74 mitogenomes: MZ047612 - MZ047685.

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