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Colonizing the Wild West: Low Diversity of Complete Mitochondrial Genomes in Western North Pacific Killer Whales Suggests a Founder Effect

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Abstract

In the North Pacific, fish-eating R-type "resident" and mammal-eating T-type "transient" killer whales do not interbreed and differ in ecology and behavior. Full-length mitochondrial genomes (about 16.4 kbp) were sequenced and assembled for 12 R-type and 14 T-type killer whale samples from different areas of the western North Pacific. All R-type individuals had the same haplotype, previously described for R-type killer whales from both eastern and western North Pacific. However, haplotype diversity of R-type killer whales was much lower in the western North Pacific than in the Aleutian Islands and the eastern North Pacific. T-type whales had 3 different haplotypes, including one previously undescribed. Haplotype diversity of T-type killer whales in the Okhotsk Sea was also much lower than in the Aleutian Islands and the eastern North Pacific. The highest haplotype diversity for both R- and T-type killer whales was observed in the Aleutian Islands. We discuss how the environmental conditions during the last glacial period might have shaped the history of killer whale populations in the North Pacific. Our results suggest the recent colonization or re-colonization of the western North Pacific by small groups of killer whales originating from the central or eastern North Pacific, possibly due to favorable environmental changes after the Last Glacial Maximum.

Keywords: cetacean, killer whale, Last Glacial Maximum, mitogenome, North Pacific

Geographic patterns of genetic diversity are important for understanding the history of species vicariance and range expansion. This is especially true for cetaceans, because their ability to cover large distances and the lack of obvious physical barriers in the open sea make their phylogenetic history much less obvious than that of terrestrial mammals.

The killer whale, *Orcinus orca*, has a world-wide distribution but specific local forms or ecotypes occur in many regions (Ford et al. 1998; Saulitis et al. 2000; Pitman and Ensor 2003). In the North Pacific, 3 distinct reproductively isolated ecotypes have been described: fish-eating "residents" (Bigg 1982; Ford et al. 1998), mammal-eating "transients" (Bigg 1982; Ford et al. 1998) and "offshore" killer whales that likely specialize on hunting sharks (Ford et al. 2011a).

The terms "resident" and "transient" killer whales have been used conventionally to identify killer whale ecotypes in British Columbia, because the occurrence of "resident" killer whales was more predictable, and researchers initially thought that the residency pattern was a key difference between the ecotypes (Ford et al. 2000). Subsequent studies including acoustic, genomic, and ecological approaches revealed numerous significant differences among ecotypes, including feeding preferences and genetic divergence (Ford et al. 1998). The terms "residents" and "transients" proved to be misleading, because in other species they were typically used to refer to territory owners versus vagrants (Burt 1943). Some authors suggested using the term "Bigg's killer whales" to refer to "transients" (e.g., Saulitis et al. 2015) as a tribute to Michael Bigg who first described the North Pacific ecotypes (Bigg 1982). Here, we refer to "residents" as "R-type" killer whales and to "transients" as "T-type" killer whales for the sake of clarity.

The origin and phylogenetic history of ecotypes have been actively investigated and debated in recent years (Foote et al. 2011; Foote and Morin 2015; Morin et al. 2015; Moura et al. 2015; Foote and Morin 2016). Much less attention has been paid to the history of populations within ecotypes. In the eastern North Pacific (Pacific coast of Canada and the United States), killer whales are separated into distinct populations despite the lack of obvious barriers between them (Parsons et al. 2013). In some areas, populations of the same ecotype overlap but remain reproductively isolated. The most well-known example is the so-called "Northern Resident" and "Southern Resident" populations whose ranges overlap in the coastal waters of British Columbia and Washington State (Ford et al. 2000). The Southern Resident population was heavily depleted during the period of live-captures in 1960s and has not recovered; currently, it comprises fewer than 80 whales (NOAA Fisheries 2014). Despite the small and diminishing population, genetic studies show a lack of evidence for mating outside of the Southern Resident population (Ford et al. 2011b, 2018). The Northern Resident population also overlaps with another R-type population in southeast Alaska, but relationships between them are less clear (Barrett-Lennard 2000). Further west, Parsons et al. (2013) identified multiple subpopulations throughout the Aleutian Islands: one subpopulation in the eastern and one in the central Aleutian Islands, and a third subpopulation ranging from the western Aleutians to Kamchatka and the Kuril Islands, Russia.

Until recently, studies of R-type killer whale population structure in the North Pacific have been based primarily on nuclear DNA, as the diversity of the mitochondrial control region is extremely low: only 3 mitochondrial control region haplotypes differing by only single nucleotide substitutions have been resolved from over 270 R-type killer whales representing populations ranging from Washington State to Kamchatka (Parsons et al. 2013). More recently, sequencing of the complete mitochondrial genomes from 107 skin samples of R-type killer whales described at least 13 R-type mitogenome haplotypes in the North Pacific (Morin et al. 2015). Eleven of these haplotypes were found only in the eastern and central North Pacific, and only 2 haplotypes occurred in the western North Pacific. Haplotype diversity of T-type killer whales is higher than that of R-type whales: 7 mitochondrial control region haplotypes (Parsons et al. 2013) and 18 complete mitogenome haplotypes (Morin et al. 2015) have been described in the North Pacific. Haplotype diversity of T-type killer whales in the western North Pacific was lower than in the central and eastern parts, similar to the R-type haplotype diversity pattern. However, sample sizes representing the western North Pacific were small. Only 11 R-type and 7 T-type samples originated from Russian waters, suggesting that low diversity in the western North Pacific could be an artefact due to a low sample size. In this study, we generated complete mitochondrial genome sequences for 26 additional killer whale samples from Russian waters to estimate with greater confidence the diversity of mitochondrial lineages in the western North Pacific.

Methods

Data Collection

Skin biopsy samples were obtained from killer whales by remote biopsy using a crossbow from small boats. Samples were collected during the summer months (June through August) of 2009–2016 from both R-type and T-type killer whales in different areas of the Russian Pacific (Figure 1). In the populations with known social structure, we selected samples from different social units (matrilineal groups). In the populations with unknown social structure, we selected samples obtained during different encounters.

DNA Extraction and PCR Amplification

Tissue samples were stored in 96% ethanol until the time of sample processing. Total genomic DNA was isolated from skin biopsy subsamples at the Molecular Diagnostic Center of Severtsov Institute of Ecology and Evolution RAS using Diatom DNA Prep 100 (Isogene Lab. Ltd., Moscow, Russia) or InviMag Tissue DNA (STRATEC Molecular, Germany) kits according to the protocols provided by the manufacturers.

The mitogenome was amplified in 4–5 overlapping fragments using PCR primers described in Morin et al. (2010): LR3, LR4, LR2.1, and LR2.2 for most samples, and in some cases LR4 product was amplified by 2 overlapping pieces using LR4.1 and LR4.2 primer pairs. PCR reactions were carried out using 2.5X MasHFMIX -1510 (Dialat Ltd, Moscow, Russia).

PCR products were gel purified using a silica column based gel purification kit Cleanup Standard (Evrogen JSC).

Mitogenome Sequencing

DNA sequencing was performed at Belozersky Institute for Physico-Chemical Biology (Moscow State University). Libraries for sequencing were generated using the Nextera XT (Illumina) reagent kit and sequenced on an Illumina MiSeq (MiSeq Reagent Kit V2, 500 cycles), 3 runs. The number of reads per sample varied from 26 446 to 1 116 422, and the read length was 75 + 75 bp, 151 + 151 bp, or 251 + 251 bp. We did not add phiX because the mitochondrial libraries were not low complexity and there were other high-complexity libraries sequenced on the same run.

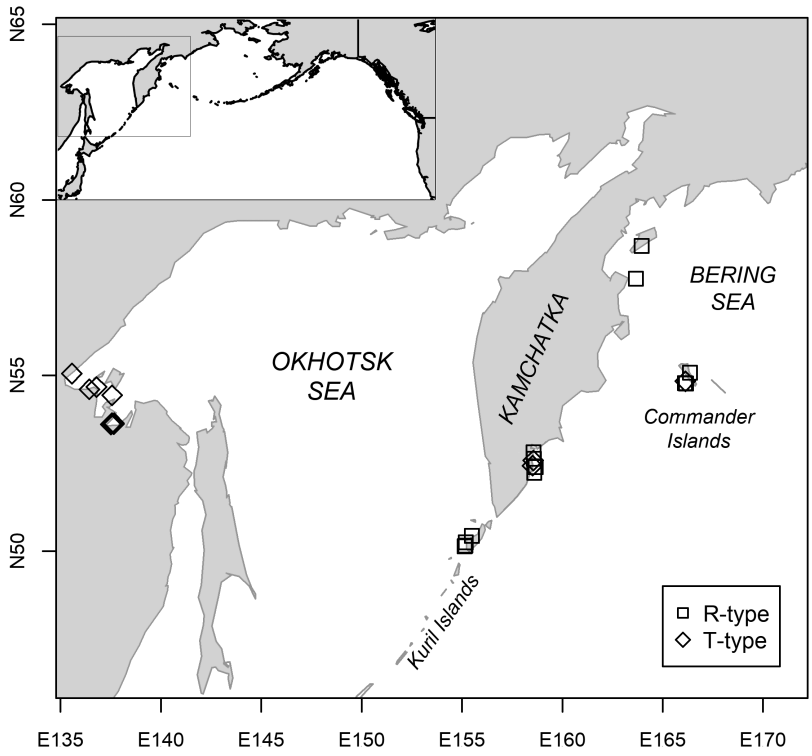

Figure 1. Locations of the killer whale samples analyzed in this study.

Sequence data were analyzed using CLC Genomics Workbench 9.5. Adapter sequences were trimmed and low quality positions (less than Q20, error probability 0.01) were removed. Sequences were mapped against the reference sequence (WNPNRRU/MtGen_66, GenBank number GU187196) with only unique mappings allowed and similarity to the reference sequence not less than 98%. Differences from the reference (SNV, single nucleotide variation) were revealed using basic variant detection with the following preferences: minimal coverage = 10, frequency not less than 90%, minimal quality = 20. Samples that had lower coverage or did not pass the frequency and quality thresholds were removed from the analysis. Consensus sequences were generated by CLC Genomics Workbench based on the reference and filtered SNVs.

Comparison to Published Data

Consensus sequences for samples from T-type whales were compared with sequences previously obtained from western North Pacific T-type killer whales (Morin et al. 2010), including WNPTRU2/MtGen_62 (GU187156), WNPTRU4/MtGen_67 (GU187157), WNPTRU1/MtGen_61 (GU187159), ENPTPI2/MtGen_44 (GU187160), WNPTRU3/MtGen_64 (GU187161) using BLASTn. Each sequence from the T-type sample was remapped on the nearest reference sequence to increase the accuracy of variant calling. The same preferences as mentioned above were used for remapping, variant detection, and filtering.

Morin et al. (2010) mentioned that for the poly-A region in the positions from 5211/5212 to 5217/5218 of the killer whale complete mitochondrial sequence, the assembly was unreliable, so the region was shortened to a fixed set of 7 As for phylogenetic analysis. All R-type and T-type sequences in Morin et al. (2015) also had 7 As in this position. We found that all our sequences (both R-type and T-type) had 8 As in this position. We used the original sequence with 8 As in this region for GenBank submission (MH062792). However, to align our sequences with Morin's, we shortened this region to 7 As for the comparative analysis to avoid introducing erroneous variation due to sequencing artifacts.

After reconciling our complete mitochondrial sequencing with those of Morin et al. (2015), we included the frequency information of samples into a single dataset for R- and T-type killer whales in the North Pacific (Table 1).

Haplotype Diversity and Network

The "haploNet" function in "pegas" library (Paradis 2010) was executed in R (R Core Team, 2015) to build a haplotype network. By default, the haplotype network is built using an infinite site model (i.e., uncorrected or Hamming distance) of DNA sequences and pairwise deletion of missing data.

Haplotype diversity in different regions was estimated using Arlequin v.3.11 software (Excoffier et al. 2007). Haplotype diversity was compared across regions using a permutation procedure implemented in R ("genetic_diversity_diffs function," Alexander et al. 2016; Alexander 2017).

Results

Full-length mitochondrial genomes of 16 387 bp were sequenced and assembled for 12 R-type and 14 T-type killer whale samples from different areas of the Russian Far East spanning more than 1000 km (Figure 1). All R-type individuals had mitochondrial genome sequences identical to that identified as MtGen_66 in Morin et al. (2015). T-type individuals had 3 different haplotypes. Two of these haplotypes have been described as MtGen_44 and MtGen_67 in Morin et al. (2015), and 1 haplotype (GenBank MH062792) has not been previously described. This haplotype was found exclusively in the Okhotsk Sea.

Table 1. Haplotype diversity (H) of R- and T-type killer whale samples from different regions of the North Pacific

Regions and localities		*N* samples	N haplotypes	H by locality	H by region
R-type killer whales					
Western NP	Kuril Islands	6	1	0.000	
	E Kamchatka	11	2	0.182	0.091
	Commander Islands	5	1	0.000	
	Aleutian Islands	30	11	0.809	0.809
Eastern NP	E Bering Sea	5	2	0.600	
	Alaska	22	2	0.455	0.678
	British Columbia/Washington	37	3	0.676	
T-type killer whales					
Western NP	Okhotsk Sea	13	2	0.282	
	E Kamchatka	6	4	0.800	0.557
	Commander Islands	2	1	0.000	
	Aleutian Islands	41	10	0.835	0.835
Eastern NP	Pribilof Islands	10	3	0.511	0.848
	Alaska	13	5	0.808	
	British Columbia/Washington	12	4	0.455	
	California	12	4	0.712	

In the previous study of mitogenome sequences generated from North Pacific killer whales (Morin et al. 2015), 10 of the 11 Russian R-type killer whales had the same complete mitochondrial genome sequence, MtGen_66, as those found in the current study; this haplotype was also found in R-type killer whales from the western Aleutian Islands, Alaska, and Washington State (Figure 2). One R-type Russian killer whale sample collected in Ozernoy Gulf off northeastern Kamchatka had another haplotype, MtGen_81, differing by 2 bp from the more common MtGen_66 (Figure 3). This haplotype did not occur in any other sample from the North Pacific.

A network reconstructed the relationships between the 13 complete mitogenome haplotypes for R-type killer whales in the North Pacific (Figure 3). The 2 most frequent and widespread haplotypes, MtGen_66 and MtGen_42, were placed in the center of the network. Haplotypes MtGen_48 (the second haplotype of the Southern Resident population from Washington State) and MtGen_65 (found in the central Aleutian Islands) were separated from MtGen_66 by 1 and 2 mutational steps, respectively. Haplotype MtGen_43 was separated by 1 step from MtGen_42 and co-occurred with it in the eastern Aleutian Islands. All other haplotypes were represented by only 1 (or 2, MtGen_70) samples. Most of them were found only in the central Aleutian Islands in the Andreanof Islands area, except for MtGen_81 found in northeastern Kamchatka (Figure 2).

T-type killer whale samples from Russian waters analyzed by Morin et al. (2015) belonged to 5 different haplotypes: MtGen_44 found in the Okhotsk Sea and eastern Kamchatka, MtGen_61, MtGen_62, and MtGen_64 in eastern Kamchatka, and MtGen_67 in the Commander Islands (Figure 4). Among our samples, we found 2 of these haplotypes: MtGen_44 in 9 samples from the Okhotsk Sea and 2 samples from eastern Kamchatka, and MtGen_67 in 1 sample from the Commander Islands. Two samples from the western Okhotsk Sea belonged to the "new" haplotype (Figure 5). With the addition of this new sequence, there are now 21 complete mitochondrial haplotypes described from T-type killer whales in the North Pacific. All of these differ by more than 65 bp from the 13 haplotypes of the R-types in the North Pacific.

The constructed haplotype network revealed several haplotype clusters among T-type killer whales (Figure 5). Most Russian haplotypes were represented by the cluster consisting of haplotypes MtGen_44, MtGen_61, MtGen_64, and the new haplotype ("New" in Figure 5). MtGen_44, central for this cluster, was also the most widespread, and found in different areas of Russian waters as well as in the Aleutian and Pribilof Islands. MtGen_61 and MtGen_64 were found only in Russian waters and represented by 1 sample each.

Another cluster, which included Russian samples, consisted of 3 haplotypes: MtGen_62, MtGen_63, and MtGen_67. MtGen_62 and MtGen_67 were found only in Russian waters, while MtGen_63, the haplotype at the center of the phylogenetic cluster, was found in the Aleutians and Prince William Sound but not in Russia.

The remaining 14 haplotypes did not occur in Russia and were found only in the eastern North Pacific from the Pribilof Islands to California.

Based on the inclusion of mitogenome haplotype frequencies from Morin et al. (2015), the highest haplotype diversity for both R- and T-type killer whales was observed in the Aleutian Islands (Table 1). No haplotype diversity was observed for the R-type whales in the Commander and Kuril Islands. Haplotype diversity of R-type killer whales from the western North Pacific (E Kamchatka, Commander, and Kuril Islands) did not differ significantly from each other, but differed significantly from the Aleutian Islands and all sites in the eastern North Pacific (Table 2). Comparison of major regions—the western North Pacific, the Aleutians, and the eastern North Pacific—also showed significant differences in haplotype diversity ($P < 0.001$ for all comparisons).

For T-type whales, diversity was very low in the Commander Islands and the Okhotsk Sea. Haplotype diversity of the Okhotsk Sea T-type whales differed significantly from the diversity in all other sites except the Commander Islands (Table 3). In eastern Kamchatka, haplotype diversity was low for R-type whales, but high for T-type whales. In the eastern North Pacific haplotype diversity was significantly lower than in the Aleutian Islands, but significantly higher than in the western North Pacific for R-type whales. For T-type whales, haplotype diversity in the Aleutians was significantly higher than in Okhotsk Sea and all eastern North Pacific sites except Alaska. Among the major regions, haplotype diversity in the western North Pacific was significantly lower, than in the Aleutians and the eastern North Pacific ($P < 0.001$ for both comparisons), but the difference between the Aleutians and the eastern North Pacific was nonsignificant ($P = 0.63$).

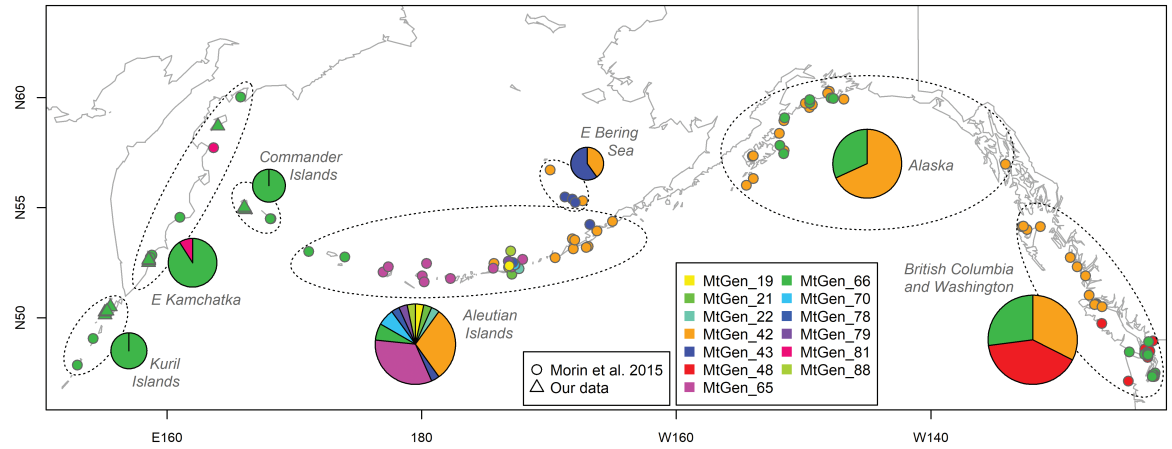

Figure 2. Distribution of complete mitochondrial haplotypes for R-type killer whales in the North Pacific. Dashed ovals indicate the predefined geographic areas, pie plots show the ratio of haplotypes in the corresponding area. The pie size is proportional to the number of individuals sampled (Table 1). See online version for full colors.

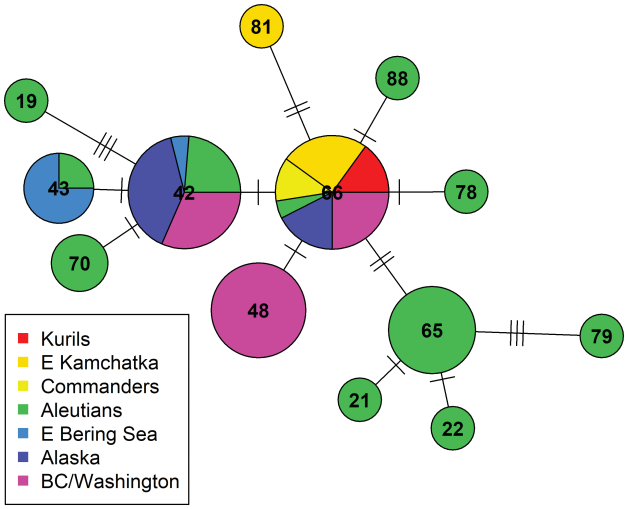

Figure 3. A network showing connections and geographical origin for the 13 mitogenome haplotypes for R-type killer whales known for the North Pacific. Numbers in the circles indicate the haplotype number. The circle size is proportional to the haplotype frequency (Table 1). Dashes indicate the number of substitutions. See online version for full colors.

Discussion

The lack of variation in complete mitochondrial genome sequences of Russian R-type killer whales is unusual, particularly considering that the samples come from different geographical regions, some situated more than 1000 km apart, and the population size is relatively large (>700 for Kamchatka, >1000 for the Commander Islands, Shabalina et al. 2015). The samples analyzed in our study and in Morin et al. (2015) cover most of the areas in the Russian Pacific where R-type killer whales are abundant (Shulezhko and Burkanov 2012); therefore, it is likely that our results are representative of the Russian Far East.

In the eastern North Pacific, different haplotypes were found even in small isolated populations, namely the Southern Resident population with <80 individuals (Morin et al. 2015). Of the 12 different mitogenome haplotypes found in the eastern and central North Pacific R-type killer whales (Morin et al. 2015), the highest diversity was observed around the Andreanof Islands in the central Aleutians. Two haplotypes were found in the eastern Aleutians, 2 in the Gulf of Alaska, and 3 in the British Columbia and Washington State area (1 in the Northern Resident and 2 in the Southern Resident populations). However, only 1 R-type haplotype was found among all R-type killer whales sampled west of 176°E both by Morin et al. (2015) and in the present study. The section of the Aleutian Islands around 176°E (Buldir Pass) was suggested by Parsons et al. (2013) to be a geographic zone of differentiation between 2 R-type killer whale subpopulations based on both nuclear microsatellite markers and mtDNA control region sequence analysis: all samples west of 176°E were found to comprise a single Russian-Western Aleutian subpopulation.

The haplotype MtGen_66 shared by the western North Pacific killer whales was not unique for this area; it was also found in many samples from the eastern North Pacific (see Supplementary Table S1 in Morin et al. 2015). Whales with this haplotype occurred in geographically separated clusters. Apart from Russia and the western Aleutians, there was 1 cluster in Alaska around Prince William Sound and Kodiak Island, and another cluster in the Washington State (Figure 2). The second most widespread haplotype, MtGen_42, was found in the eastern North Pacific from the eastern Aleutian Islands to Vancouver Island. Both of these haplotypes were positioned in the center of the haplotype network (Figure 2). The high frequency of occurrence, wide geographical distribution, and central position in the haplotype network likely indicate that these 2 haplotypes are ancestral for the North Pacific R-type killer whale population. Absence of any haplotype other than MtGen_66 in the western North Pacific (except for 1 sample in Morin et al. 2015) is probably due to a founder effect, suggesting that a small group of R-type killer whales sharing this haplotype colonized the western North Pacific relatively recently and then expanded, giving rise to all contemporary R-type killer whale communities in the Russian Pacific.

Killer whales are highly mobile and can cover hundreds of kilometers over a span of several days (Durban and Pitman 2012). As such, it is likely that some obstacle to dispersal existed in the past to have inhibited colonization of the western North Pacific by multiple matrilines. The only significant obstacle that would likely impede killer whale movements across open marine waters is the presence of sea ice. Killer whales generally avoid ice (Matthews et al. 2011) except for some Antarctic ecotypes that specialize in hunting seals among the ice floes or fish under the ice (Pitman and Ensor 2003).

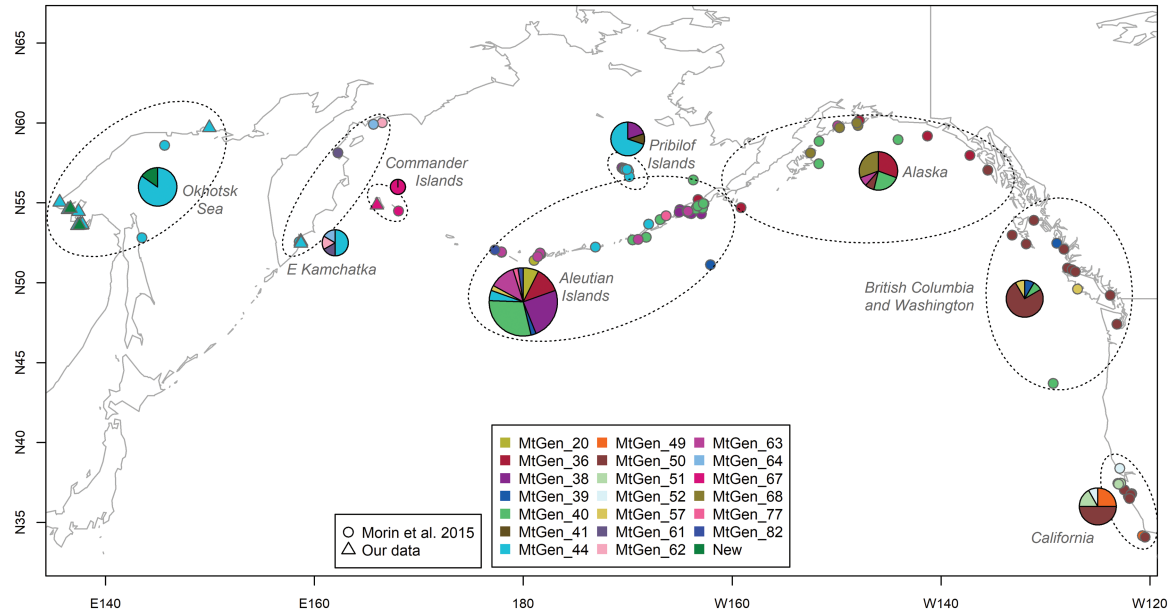

Figure 4. Distribution of complete mitochondrial haplotypes for T-type killer whales in the North Pacific. Dashed ovals indicate the predefined geographic areas, pie plots show the ratio of haplotypes in the corresponding area. The pie size is proportional to the number of individuals sampled (Table 1). See online version for full colors.

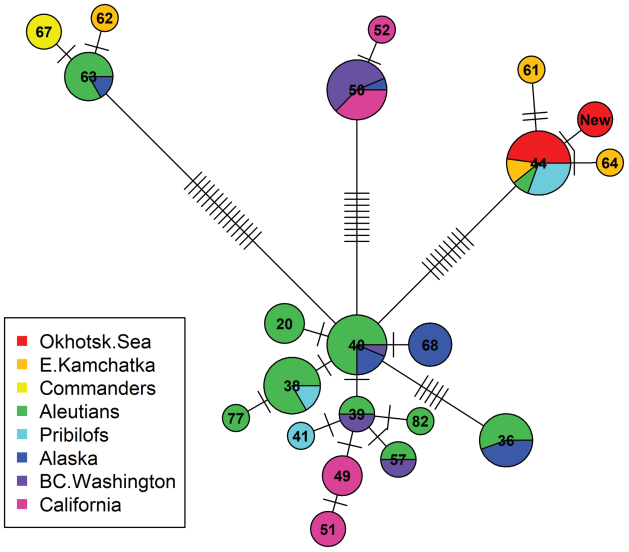

Figure 5. A network showing connections and geographical origin for 21 complete mitochondrial haplotypes of T-type killer whales in the North Pacific. The circle size is proportional to the number of individuals sampled belonging to that haplotype (Table 1). Dashes indicate the number of substitutions. See online version for full colors.

During some phases of the last glacial period, a significant ice sheet covered parts of maritime Pacific Russia. The northern and western parts of Okhotsk Sea could have had perennial sea ice coverage during the Last Glacial Maximum (Gorbarenko et al. 2010). A large Kamchatka–Koryak Ice Sheet with extensive marine-terminating ice margins possibly existed during the last glacial period (Bigg et al. 2008). Both R- and T-type killer whales have a primarily coastal distribution (Forney and Wade 2006), so even coastal glaciation could effectively prevent colonization of these regions.

Ice sheets also existed in Alaska and British Columbia during the Wisconsin Glaciation (Blaise et al. 1990) which should have also moved killer whales away from the region. Indeed, the haplotype diversity along the west coast of North America is lower compared with the Aleutian Islands, though still higher than in the western North Pacific. To avoid the Wisconsin ice sheet, killer whales could have retreated either west (to the Aleutian Islands) or south (to California). The small Southern Resident population, currently ranging from British Columbia to California (NOAA Fisheries 2014), could be the descendants of the killer whales that retreated to the south. This might explain their lack of gene flow with partially sympatric Northern Residents, which appear to be more related to the Alaskan Residents by nuclear markers (Barrett-Lennard 2000) and could be the descendants of the whales that survived in the Aleutian Islands during glaciation.

The central Aleutian Islands were mostly ice-free during the Last Glacial Maximum (Katsuki and Takahashi 2005) and served as a refugium for several local species. Holder et al. (1999, 2000) suggested that 2 currently unidentified refugia in the Bering Sea region may have existed during the Wisconsin, based on the unexpectedly high genetic diversity of rock ptarmigan within the Aleutian Islands relative to that across mainland Alaska and eastern Siberia. Pruett and Winker (2008) also concluded that populations of rock sandpiper, rock ptarmigan, common raven, and winter wren in the Aleutian and Commander Islands were isolated in the same refugia from their mainland counterparts during the late Pleistocene.

In the western North Pacific, areas at the south of the current R-type killer whale range (Kuril Islands and southern Okhotsk Sea) were more or less blocked by perennial floating ice (Takahashi 1998). During periods of ice formation, killer whales may have responded by retreating east to the Aleutian Islands or south to Japan. Analyzing samples from Japanese R-type whales would be an important next step in further elucidating the history of killer whale populations in the North Pacific.

Table 2. Results of the test for significant differences in haplotype diversity of R-type killer whales from different regions

	Kuril Islands	E Kamchatka	Commander Islands	Aleutian Islands	E Bering Sea	Alaska	British Columbia/ Washington
Kuril Islands	—	0.066	1.000	0.000*	0.001*	0.000*	0.000*
E Kamchatka	0.182	—	0.125	0.000*	0.008*	0.001*	0.000*
Commander Islands	0.000	0.182	—	0.000*	0.011*	0.009*	0.000*
Aleutian Islands	0.809*	0.627*	0.809*	—	0.056	0.000*	0.001*
E Bering Sea	0.600*	0.418*	0.600*	0.209	—	0.118	0.462
Alaska	0.455*	0.273*	0.455*	0.355*	0.145	—	0.000*
British Columbia/ Washington	0.676*	0.494*	0.6768	0.134*	0.076	0.221*	—

Haplotype diversity differences are shown below diagonal, *P* values are shown above diagonal. Asterisks (*) indicate $P \leq 0.05$ based on 1000 random permutations of the original data set.

Table 3. Results of the test for significant differences in haplotype diversity of T-type killer whales from different regions

	Okhotsk Sea	E Kamchatka	Commander Islands	Aleutian Islands	Pribilof Islands	Alaska	British Columbia/ Washington	California
Okhotsk Sea	—	0.003*	0.105	0.000*	0.011*	0.000*	0.024*	0.000*
E Kamchatka	0.518*	—	0.088	0.621	0.025*	0.923	0.009*	0.341
Commander Islands	0.282	0.800	—	0.094	0.101	0.093	0.101	0.095
Aleutian Islands	0.553*	0.035	0.835	—	0.001*	0.527	0.000*	0.031*
Pribilof Islands	0.229*	0.289*	0.511	0.324*	—	0.002*	0.412	0.022*
Alaska	0.526*	0.008	0.808	0.028	0.297*	—	0.000*	0.140
British Columbia/ Washington	0.172*	0.345*	0.455	0.381*	0.057	0.353*	—	0.005*
California	0.430*	0.088	0.712	0.123*	0.201*	0.096	0.258*	—

Haplotype diversity differences are shown below diagonal, *P* values are shown above diagonal. Asterisks (*) indicate $P \leq 0.05$ based on 1000 random permutations of the original data set.

Decreased diversity of maternal lineages in the western North Pacific was also found for harbour porpoise *Phocoena phocoena* (Taguchi et al. 2010). The authors suggest that this decrease was caused by the Late Pleistocene climatic changes which might have affected both the habitat suitability for porpoises and the distribution and abundance of their prey. The present western North Pacific porpoise population presumably originated in the middle of Late Pleistocene from a few founders coming from a refugium at the northeastern edge of the porpoise range (Gulf of Alaska or Bering Sea).

Marine ice cover plays an important role in heat and gas exchange between sea surface and atmosphere, with a strong influence on phytoplankton ecology and primary productivity. In the glacial western North Pacific, productivity was most likely low (Narita et al. 2002). Ice sheets and a colder climate during the glaciation periods likely also reduced the abundance of salmon (McPhail and Lindsey 1986), the primary prey of contemporary R-type killer whales in most regions (Saulitis et al. 2000; Ford and Ellis 2006; Hanson et al. 2010). To survive during glacial periods, killer whales most likely adopted a strategy of either prey switching or moving to more productive regions, which, along with the ice avoidance, may have also contributed to the retreat of R-type killer whale populations during the last glacial period.

In T-type killer whales, haplotype diversity was also higher in the Aleutian Islands, but the reduction of diversity in the coastal eastern and western North Pacific was less pronounced than in R-type killer whales. The lowest diversity was observed in the Commander Islands, where both samples had the same haplotype, and in the Okhotsk Sea, where only 2 haplotypes were found in 13 samples, which is consistent with the proposed ice-driven relocation of killer whales from this area during the last glacial period. Haplotype diversity was found to be higher in eastern Kamchatka and along coastal North America in the eastern North Pacific suggesting that these areas may have retained some populations of T-type killer whales during glaciation. These populations may have survived because they depended on pagophilic seals, which thrived in ice-covered areas in contrast to salmon (McPhail and Lindsey 1986).

The pattern of diversity of the complete mitochondrial genomes of R-type killer whales in the eastern and the western North Pacific is similar to the diversity of killer whale dialects in these areas. Killer whale dialects are vertically transmitted between generations through social learning (Deecke et al. 2000; Foote et al. 2006; Crance et al. 2014) and therefore change faster than DNA sequences. The process of cultural evolution of killer whale dialects parallels genetic evolution, so dialect similarity can serve as an independent marker of the phylogenetic history of populations. Indeed, Filatova et al. (2012) found that the diversity of biphonic calls was the lowest in Kamchatkan R-type killer whales from the western North Pacific when compared with 3 populations from Alaska, British Columbia, and Washington State. Danishevskaya and Filatova (2017) also found that the calls of the western North Pacific R-type killer whales were more similar to each other, than the calls of any of the 3 eastern North Pacific populations.

The lack of diversity in the mitochondrial genomes and acoustic dialects of the western North Pacific R-type killer whales also fits with the hypothesis of cultural hitchhiking (Whitehead et al. 2017). This hypothesis suggests that selection on cultural traits can reduce the diversity of genes that are being transmitted in parallel with the culture. R-type killer whales have a matrilineal social structure whereby both female and male offspring remain in their natal group for their entire lives (Ford et al. 2000). Therefore, they acquire both

mitochondrial DNA and cultural traditions from their mothers. If a group of killer whales developed a novel cultural tradition that increased its fitness, this population could have expanded over the western North Pacific, either replacing groups with less adaptive traditions, or colonizing new uninhabited areas. As a result, the distribution of their mitochondrial DNA haplotype would also expand over this area.

In summary, our results suggest the recent colonization of the western North Pacific by a small group of R-type killer whales originating from the central or eastern North Pacific, which is also supported by the analysis of acoustic dialects. The recent recolonization of the Okhotsk Sea by T-type killer whales after the Last Glacial Maximum also appears to be the likely scenario. The available data do not enable the reconstruction of the full history of killer whale expansion over the North Pacific. More focused research is needed to explain the geographic patterns of genetic variability in this area.

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Data Availability

We have deposited the primary data underlying these analyses as follows:

- Sampling locations and mitogenome genotypes to Dryad (doi:10.5061/dryad.c003f8c)
- DNA sequences: Genbank accession MH062792.

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