

Geographic Patterns of Genetic Differentiation among Killer Whales in the Northern North Pacific

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Abstract

The difficulties associated with detecting population boundaries have long constrained the conservation and management of highly mobile, wide-ranging marine species, such as killer whales (*Orcinus orca*). In this study, we use data from 26 nuclear microsatellite loci and mitochondrial DNA sequences (988 bp) to test a priori hypotheses about population subdivisions generated from a decade of killer whale surveys across the northern North Pacific. A total of 462 remote skin biopsies were collected from wild killer whales primarily between 2001 and 2010 from the northern Gulf of Alaska to the Sea of Okhotsk, representing both the piscivorous “resident” and the mammal-eating “transient” (or Bigg’s) killer whales. Divergence of the 2 ecotypes was supported by both mtDNA and microsatellites. Geographic patterns of genetic differentiation were supported by significant regions of genetic discontinuity, providing evidence of population structuring within both ecotypes and corroborating direct observations of restricted movements of individual whales. In the Aleutian Islands (Alaska), subpopulations, or groups with significantly different mtDNA and microsatellite allele frequencies, were largely delimited by major oceanographic boundaries for resident killer whales. Although Amchitka Pass represented a major subdivision for transient killer whales between the central and western Aleutian Islands, several smaller subpopulations were evident throughout the eastern Aleutians and Bering Sea. Support for seasonally sympatric transient subpopulations around Unimak Island suggests isolating mechanisms other than geographic distance within this highly mobile top predator.

Key words: ecotypes, genetic structure, mtDNA, microsatellite, *Orcinus orca*, populations, subpopulations

Population boundaries are often difficult to define for highly mobile species with largely continuous geographical distributions. However, identifying patterns of population structure is critical for the effective management and conservation of natural populations, and for identifying subpopulations requiring unique management strategies (Awise 1994). Furthermore, underlying population genetic structure has considerable evolutionary and ecological relevance, providing unique insight into mechanisms of reproductive isolation and patterns of localized adaptation, and furthering our understanding of the factors that shape these subdivisions

and drive divergence. Beyond population delimitation and identification of stock boundaries, understanding patterns of gene flow and dispersal is fundamental for evaluating population status.

High mobility and dispersal capabilities, combined with a seemingly homogenous marine habitat, were initially assumed to translate into high levels of gene flow within oceanic species (Palumbi 1994). Analytical advances have provided the tools necessary to directly examine geographic structuring among individual animals, and recent studies of a variety of marine vertebrate species have clearly demonstrated that high

potential mobility cannot be used as a predictor of effective gene flow (Carreras et al. 2007; Verissimo et al. 2010; Sandoval-Castillo and Rocha-Olivares 2011). Despite the lack of obvious physical barriers to dispersal and gene flow, molecular genetic studies of many species within the taxonomic order Cetacea have clearly dispelled the assumption of panmixia, documenting numerous cases involving significant geographic patterns of population genetic differentiation (Baker et al. 1998; Rosel et al. 1999; Parsons et al. 2006; Fontaine et al. 2007; Mirimin et al. 2009; Rosenbaum et al. 2009). Because cetaceans are marine predators with remarkable longevity and both direct and indirect interactions with commercial fisheries, understanding the structuring of their populations has important implications for understanding ecosystem processes on both local and global scales.

The killer whale (*Orcinus orca*), a large, globally distributed delphinid, is among the better known of cetacean species. In the northeastern Pacific, long-term studies on several small populations of piscivorous killer whales have contributed unprecedented insight into their habits, social organization, philopatry to matrilineal groups and, more recently, patterns of gene flow (Balcomb and Bigg 1986; Bigg et al. 1990; Parsons et al. 2009; Ford et al. 2011). Studies focusing on the behavioral ecology of killer whales have identified 3 divergent yet sympatric ecotypes inhabiting northern North Pacific waters (Bigg 1982; Ford et al. 1998). The 3 ecotypes (commonly referred to as “residents,” “transients,” or Bigg’s killer whales, in tribute to the late Dr Michael Bigg (Ford 2011; Riesch et al. 2012), and “offshores”) differ phenotypically and show marked differences in patterns of dispersal, acoustic patterns, social structure, group dynamics, and prey preferences (Baird and Stacey 1988; Bigg et al. 1990; Ford 1991; Barrett-Lennard et al. 1996; Ford et al. 1998; Baird and Whitehead 2000; Foote and Nystuen 2008; Ford et al. 2011). In addition to the genetic differences among ecotypes first described by Stevens et al. (1989) and Hoelzel and Dover (1991), recent analyses of the entire mitochondrial genome suggested that some of the unique killer whale ecotypes represent deeply divergent evolutionary lineages and warrant elevation to species or subspecies status (Morin et al. 2010). For example, estimates from mitogenome sequence data indicate that transient killer whales diverged from all other killer whale lineages some 700 000 years ago, and the ad hoc committee on marine mammal taxonomy currently recognizes the 2 predominant North Pacific ecotypes as unnamed *Orcinus orca* subspecies (Committee on Taxonomy 2012). Coalescent analyses further suggest that the ecological divergence between the resident and transient ecotypes may have arisen during an allopatric period preceding the migration of ancestral resident maternal lineages back into the North Pacific resulting in secondary contact and the current sympatric distribution (Foote et al. 2011). The broad distribution of killer whales throughout coastal and offshore waters, combined with its ecological specializations, presents an ideal opportunity to compare patterns of genetic structuring among ecotypes and contrast the socioecological factors that shape patterns of gene flow and population structuring.

As a result of multiple decades of individual-based studies, population structure is well characterized for killer whales around Prince William Sound/Kenai Fjords, in the coastal waters of the Gulf of Alaska (Matkin 1997; Matkin et al. 1999), and for those inhabiting the coastal waters further south around British Columbia and Washington State (Bigg et al. 1990; Ford 1991; Baird and Whitehead 2000; Ford et al. 2011). However, less information is available for whales inhabiting waters of the western Gulf of Alaska, Aleutian Islands, Bering Sea, and Russia. Despite a relatively ubiquitous distribution, data documenting individual movements and social affiliations (Durban et al. 2010; Fearnbach 2012), as well as telemetry data (Durban J, unpublished data; Matkin et al. 2012) suggest that some individuals and matrilineal pods exhibit restricted movements and a high degree of interannual site fidelity. However, contemporary estimates of gene flow are lacking for these northern areas, and documented movements of individual whales between Kodiak Island and southeastern Alaska, for example, suggest a certain degree of connectedness (Matkin 1997; Matkin et al. 1999, 2012). As a consequence of the uncertainty surrounding the population structuring within these regions and a lack of data for the westernmost reaches of the northern North Pacific, current stock designations encompass very broad areas. According to the stock assessment requirements of the US Marine Mammal Protection Act (MMPA), resident killer whales inhabiting the waters in the far North Pacific are currently recognized as a single stock ranging from southeast Alaska through the Aleutian Islands and Bering Sea (Allen and Angliss 2011). The US MMPA stock designation for transient killer whales recognizes 2 stocks with overlapping geographic distributions, comprising the “Aleutian and western” stock (Gulf of Alaska, Aleutian Islands, and Bering Sea), and the much smaller community of “AT1” killer whales whose range appears to be largely restricted to Prince William Sound and the Kenai Fjords (Allen and Angliss 2011; Matkin et al. 1999). Recent work examining the social structure of resident killer whales within the “Alaska resident stock” described social networks that are spatially connected yet exhibit differential ranging patterns (Fearnbach 2012). Such socially mediated spatial structuring may provide a basis for population genetic subdivisions similar to that described for the Northern and Southern resident killer whale communities off the coast of British Columbia and Washington State (Ford et al. 2000).

As apex predators with high energetic requirements (Noren 2011; Williams et al. 2004, 2011), killer whales are of both management and conservation concern throughout the North Pacific. Predation on, and competition with, both endangered and commercially important species (e.g., marine mammals, salmonids) make killer whales a species of interest throughout Alaskan waters and beyond. In this study, we use both mitochondrial (mtDNA) sequences and nuclear (nDNA) microsatellite genotypes to examine genetic structure of 2 ecotypes (residents and transients) within the genus *Orcinus* in northern North Pacific waters. The patterns of genetic discontinuities resolved in this study will provide data to support a revision of stock structure in the North

Pacific and provide insight into some of the ecological factors shaping killer whale populations.

Methods

DNA Extraction and PCR Amplification

Skin biopsy samples were obtained from killer whales by remote dart biopsy (Barrett-Lennard et al. 1996; Parsons et al. 2003) during dedicated and opportunistic shipboard surveys across the North Pacific. Samples were collected primarily during the summer months (June through August), primarily between 2001 and 2010 from both resident and transient killer whales (Table 1). Tissue samples were stored frozen in 99% ethanol or salt-saturated dimethyl sulfoxide solution until the time of sample processing. Total genomic DNA was isolated from skin biopsy subsamples using a variety of common extraction methods, including silica-based filter membranes (Qiagen, Valencia, CA), standard phenol/chloroform extraction (modified from Sambrook et al. 1989), and lithium chloride (Gemmell and Akiyama 1996). DNA concentrations were determined by absorbance on a NanoDrop ND-8000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and normalized to a working concentration of 2 ng/ μ L. Remaining skin biopsy fragments and extracted DNA were archived at -80°C .

The mitochondrial control region was amplified via polymerase chain reaction (PCR) in 20 μ L reaction volumes as described in Zerbini et al. (2007). Both strands of the amplicon were sequenced independently using Applied Biosystems (ABI, Carlsbad, CA) BigDye Terminator v3.1 Cycle Sequencing Kit on the ABI model 3100 sequencer. Sequences were manually checked for sequencing errors or questionable base calls and aligned using ClustalW (Thompson et al. 1994) as implemented in BioEdit (Hall 1999). Control region haplotypes were assigned based on comparison with previously

published killer whale sequences deposited in GenBank. Haplotypic (h) and nucleotide (π) diversities were estimated according to Nei (1987) to describe the control region sequence divergence and haplotype frequency differences using Arlequin v3.11 (Excoffier et al. 2005).

Samples were genotyped at 27 polymorphic microsatellite loci (see Supplementary Appendix 1 online). Initially, each locus was amplified individually in 10 μ L reactions containing 4 ng of genomic DNA, 1X Promega GoTaq Flexi Buffer, 2.5 mM MgCl_2 , 0.2 mM of each dNTP, 0.1 $\mu\text{g}/\mu\text{L}$ of bovine serum albumin, 0.2 μM of each primer (forward primers were fluorescently labeled), and 0.5 units of GoTaq Flexi DNA Polymerase (Promega, Madison, WI). Thermocycler profiles included initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 35 s, T_a $^{\circ}\text{C}$ for 35 s, 72°C for 35 s, and a final extension at 72°C for 30 min. Amplification conditions were further optimized, and the majority of loci were multiplexed as groups of 2–4 loci with nonoverlapping allele sizes using the Qiagen Multiplex PCR Kit. Each multiplex PCR was performed according to the conditions suggested by Qiagen Multiplex PCR Kit handbook in a total reaction volume of 20 μ L. Additional PCR conditions are described in Supplementary Appendix 1 online. Amplified products were analyzed using an ABI 3100 automated DNA sequencer, and allele sizes were determined using ABI LIZ500 as the internal size standard. ABI GeneScan v3.7 and Genotyper v3.7 (ABI) software were used to collect and analyze microsatellite data.

Genotyping quality control measures included negative control reactions at each step including DNA extraction, PCR, and sequencing, as well as replicate genotyping of multiple samples. An overall genotyping replication rate of $\geq 11\%$ of samples allowed us to empirically estimate the per-allele genotyping error rate (Hoffman and Amos 2005; Morin et al. 2010). Furthermore, each PCR set included at least 2 samples previously genotyped to provide cross-plate controls and ensure consistent allele binning throughout the study.

Table 1 Sample sizes across a priori strata for both resident and transient killer whales sampled across the northern North Pacific

Geographic region	Ecotype: a priori stratum	Collection years	Number of samples		
			Total	Resident	Transient
Central Aleutians	RES-CAL	2001–2010	61	61	
Eastern Aleutians	RES-EAL	1997–2010	56	56	
Gulf of Alaska	RES-GOA	2001–2005	32	32	
Russia	RES-RUS	1994–2006	117	117	
Western Aleutians	RES-WAL	2004–2010	8	8	
Eastern Aleutians	TRANS-EAL	1990–2009	44		44
Unimak Island	TRANS-UI	2001–2009	16		16
Gulf of Alaska	TRANS-GOA	2004	13		13
Kamchatka Peninsula	TRANS-KAM	2002–2006	11		11
Kodiak Island	TRANS-KOD	2001–2005	7		7
Sea of Okhotsk	TRANS-OKH	2001–2004	6		6
Pribilof Islands	TRANS-PRI	2005–2009	30		30
Rat Island Group	TRANS-RAT	2006–2010	11		11
Tanaga Island	TRANS-TAN	2003–2010	5		5

Counts reflect the number of individually genotyped whales after the removal of genetically identical biopsies.

Ecotype Identification and Genetic Assignment

Ecotype identification for each sample was based on both photographic identification of individuals using phenotypically distinctive characteristics of whales in sampled groups and mitochondrial control region sequence (Matkin et al. 2007; Zerbinì et al. 2007; Durban et al. 2010). The ability to reliably identify ecotype based on characteristic pigmentation and morphological differences (Baird and Stacey 1988; Ford et al. 2000) and fixed mtDNA sequence differences (Hoelzel et al. 1998; Barrett-Lennard 2000; Hoelzel et al. 2002) has been previously demonstrated for North Pacific killer whales (Zerbinì et al. 2007). For the 6 samples in the data set for which the above data were unavailable, ecotype was identified post hoc by examining the clustering of samples in a principal coordinate analysis (PCA) based on multilocus data and by individual assignment tests as executed in GeneClass (see below).

The probability of an individual belonging to a particular ecotype was estimated using the Bayesian assignment method of Rannala and Mountain (1997) as implemented in GeneClass v.2 (Piry et al. 2004), and the clustering algorithms implemented in STRUCTURE (Pritchard et al. 2000), run naively without the inclusion of prior information on ecotype or location (see below for model specifics). The clustering of individual samples according to pairwise genotypic distance was examined using PCA as implemented in GenAlEx v.6.4 (Peakall and Smouse 2006). Genetic differentiation (nDNA) between resident and transient ecotypes was estimated using both F_{ST} (Weir and Cockerham 1984) and F'_{ST} (Hedrick 2005), calculated using custom code (Mesnick et al. 2011) written in the statistical programming language R (R Development Core Team 2011). Arlequin v.3.5.1.2 (Excoffier and Lischer 2010) was used to estimate both F_{ST} and Φ_{ST} (Tamura and Nei, 1993; $\alpha = 0.5$) for mtDNA sequence data. Statistical significance for all metrics was determined by 10 000 random permutations of the original data set.

Identifying Duplicate Samples, Estimating Genetic Diversity, and the Removal of Close Kin

Microsatellite Toolkit (Park 2001) and GENECAP (Wilberg and Dreher 2004) were used to examine the microsatellite genotype data set for potential errors and to identify duplicate genotypes by comparing each multilocus genotype to all others in the data set. All pairs of genotypes that mismatched at 3 or fewer loci were rechecked for potential scoring errors by re-examining the electropherograms for those loci. Pairs of samples that were identified as genetic matches were further examined by comparing associated field (photographic identifications) and molecular (control region haplotypes and genetic sex) data. GENECAP (Wilberg and Dreher 2004) was also used to calculate the probability of identity ($P_{(ID)}$): the probability that 2 unrelated individuals share the same multilocus genotype by chance. The observed $P_{(ID)}$ was calculated using the more traditional formula assuming Hardy–Weinberg equilibrium (HWE; Paetkau and Strobeck 1994), as well as the conservative estimator of $P_{(ID)}$ for full siblings ($P_{(ID)sib}$; Waits et al. 2001). Estimates for $P_{(ID)sib}$ were used to

empirically assess a minimum threshold for the number of loci genotyped by calculating $P_{(ID)sib}$ for increasing numbers of loci. Including data from the least heterozygous loci first, we derived a conservative estimate of the minimum number of loci needed to identify individual whales and achieve a probability of identity for siblings ≤ 0.001 (Waits et al. 2001).

After removal of duplicate samples from the data set, genetic diversity within each ecotype was quantified as the mean number of alleles per locus (N_a), allelic richness (AR), observed (H_o) and expected heterozygosity (H_e), and inbreeding coefficient (F_{IS}) using FSTAT (Goudet 2000) and GenAlEx (Peakall and Smouse 2006). Departures from HWE expectations using the Fisher's Exact test (Guo and Thompson 1992) and tests for genotypic disequilibrium among the loci were assessed using GENEPOP v4.0 (Raymond and Rousset 1995). Multiple tests error rate was adjusted using the sequential Bonferroni correction (Rice 1989).

Data sets containing a large number of closely related individuals have the potential to impact estimates of population structure and inflate measures of genetic distance through violations of model assumptions due to allelic enrichment (Amos et al. 1993). Long-term studies of several killer whale populations have documented extreme philopatry to natal groups and a matrifocal social organization within populations (Balcomb and Bigg 1986; Bigg et al. 1990; Ford et al. 1994; Matkin et al. 1999; Parsons et al. 2009). Because the focus of this study is to examine population structure on a fairly broad scale geographically, we addressed potential kin bias by estimating pairwise relatedness within each ecotype from microsatellite allele frequency data. KINGROUP (Konovalov et al. 2004) was used to estimate pairwise relatedness according to Lynch and Ritland's (1999) regression-based estimator (R_{LR}). Relatedness estimates were compared with the maximum value obtained from a simulated set of 10 000 pairs of unrelated individuals (UR) using the observed allele frequencies. Pairs of individuals with $R_{LR} > UR_{MAX}$ were considered to be potential close relatives and 1 individual from the pair was removed for analyses of spatial genetic patterns to minimize the impact of inclusion of kin in the data set.

Testing a priori Hypotheses of Geographic Structure

Geographic structure was first examined by testing a priori subdivisions. Putative geographic strata were defined based on data acquired from georeferenced photographic records of individual killer whales (Wade P, Durban J, unpublished data; Durban et al. 2010), the geographic extent of social network clusters (Fearnbach 2012), and the presence of large geophysical barriers (e.g., Kamchatka peninsula). Strata names were based on general geographic regions, and samples were assigned to the stratum in which they were sampled. These assignments are not intended to convey core areas for individually sampled killer whales. Despite some long-range movements of individual whales, social network analyses highlight a strong spatial component that was used to inform a priori strata (Fearnbach 2012). However, individual sighting histories were limited for the majority of killer whales encountered in the Aleutian Islands and Bering

Sea. Therefore, with the exception of transient killer whales comprising the Unimak Island stratum (see below), spatial genetic structure was tested by assigning individual whales to the stratum in which they were sampled.

Resident killer whales were assigned to 5 large a priori subdivisions delimiting putative populations that were arranged largely along longitudinal lines and significant oceanographic boundaries in the North Pacific (Figure 1a): Russia (RUS), western Aleutian Islands (WAL), central Aleutians (CAL), eastern Aleutians (EAL), and the Gulf of Alaska (GOA). Transient killer whales were assigned to 9 smaller putative subdivisions: Sea of Okhotsk (OKH), Kamchatka peninsula (KAM), the Rat Islands group (RAT), Tanaga Island (TAN), Pribilof Islands (PRI), eastern Aleutian

Islands (EAL), Unimak Island (UI), Kodiak Island (KOD), and the Gulf of Alaska (GOA) (Figure 2a). In the eastern Aleutians, samples were assigned to the Unimak Island (UI) stratum based on behavioral data documenting the presence of identified whales in spring killer whale assemblages foraging on migrating gray whales (Barrett-Lennard et al. 2011; Durban et al. 2010). A priori hypotheses about population structure were first tested by estimating both nuclear and mitochondrial genetic differentiation among these strata. Measures of genetic differentiation including pairwise measures of F_{ST} (Weir and Cockerham 1984), F'_{ST} (Hedrick 2005), G'_{ST} (Hedrick 2005; Meirmans and Hedrick 2010) and chi square were calculated from nuclear microsatellite data using the custom R code as described above. Both F_{ST} and

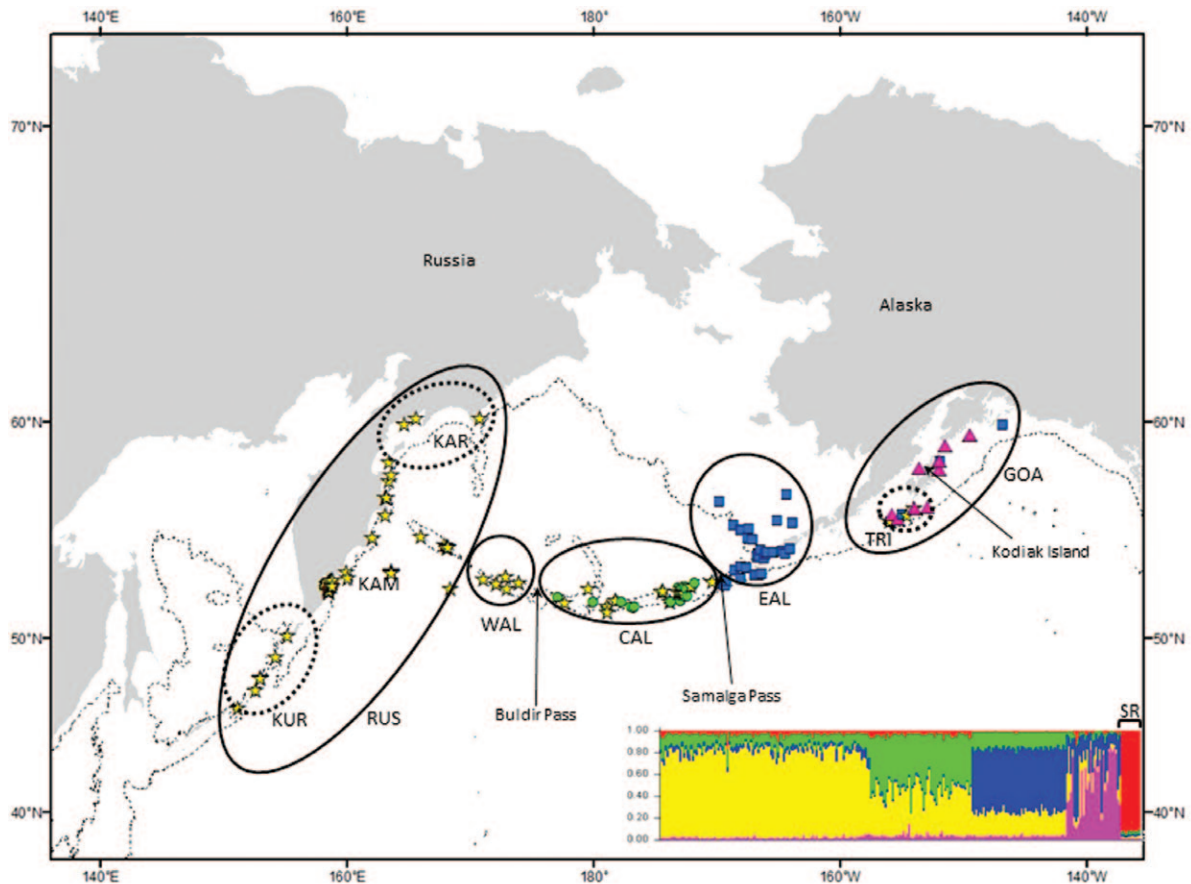


Figure 1. Resident killer whale samples included in this study plotted according to biopsy sample locations. (a) Solid line ellipses indicate the extent of a priori geographic strata. Dotted lines surround putative strata indicated by Wombling analyses and included in pairwise tests of genetic differentiation. Symbols representing individual samples are colored according to the STRUCTURE cluster (model for $k = 5$) to which they were assigned with the highest probability (mean \pm SD = 0.677 ± 0.143). Inset figure shows the STRUCTURE bar plot ($k = 5$), where each vertical bar represents the proportional membership of individual whales within each of inferred genetic clusters, individuals are ordered by longitude. Samples representing the southern resident killer whale population (“SR” on the far right of the inset plot) sampled in Washington State are not mapped. (b) Ellipses indicate a posteriori geographic strata based on analysis of nDNA and mtDNA data. Individual samples are coded according to control region mtDNA haplotype. Inset figure shows regions of genetic discontinuity (light grey) identified by WOMBOSOFT indicating significant putative genetic boundaries for resident killer whales. The 1000 m bathymetric depth contour is indicated by a thin broken line.

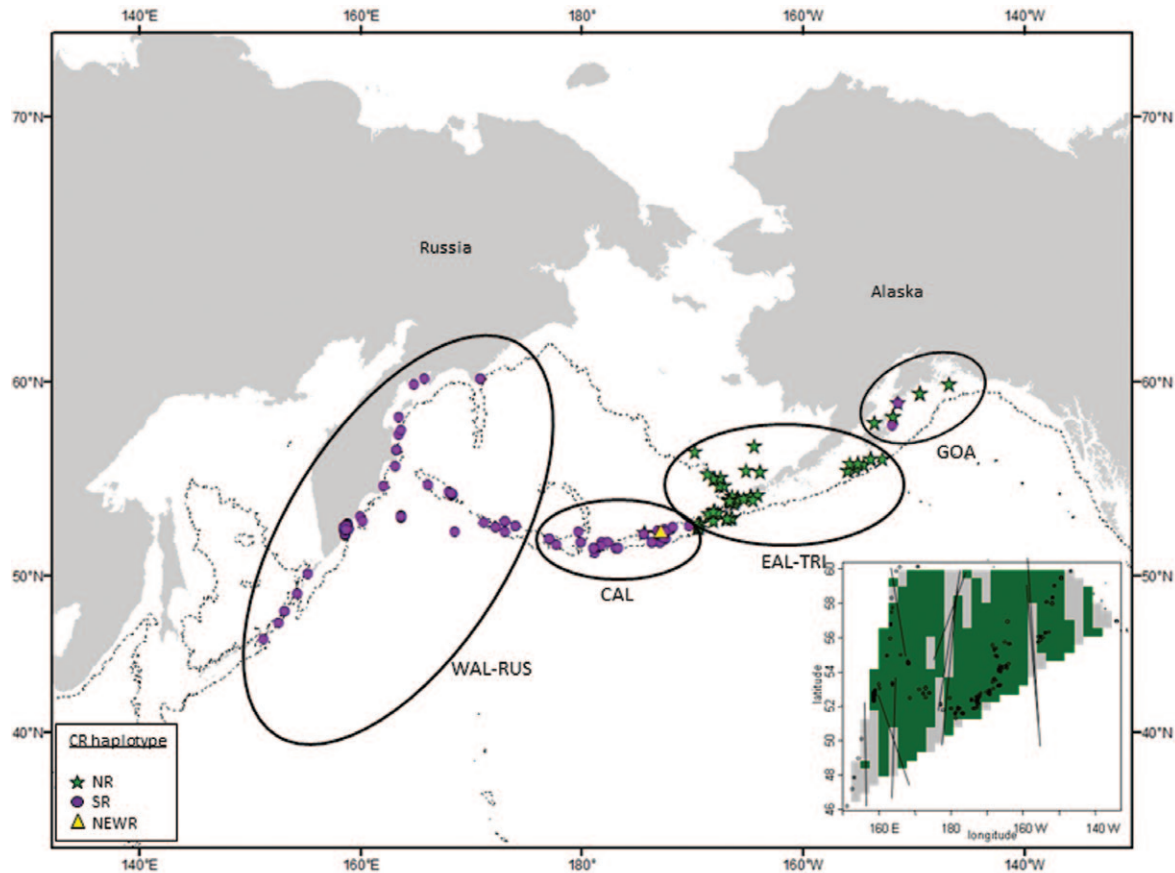


Figure 1. Continued

Φ_{ST} overall, and for all pairwise comparisons among strata, were estimated as above for mtDNA sequence data.

Detecting Spatial Genetic Clusters

The presence of spatial genetic discontinuities or population boundaries that were not reflected by the a priori subdivisions was explored using 2 complementary methods. First, the Wombling method was applied as implemented in the R package, WOMBOSFT (Crida and Manel 2007). This method uses geographically referenced individual genotypes to compute allele frequencies across the study region, and calculates the gradient of these surfaces to infer genetic boundaries between populations (Zhu et al. 2011). Default values were used for the WOMBOSFT models, with the exception of the grid size that was set at 30×30 across the entire study area and a bandwidth of $b = 1.0$. Longitudes were manually transformed to avoid negative values east of 180° , facilitating interpretation of the resulting candidate boundaries map. Statistical significance of genetic boundaries was assessed at a level of $\alpha = 0.05$.

The Bayesian clustering algorithm implemented in STRUCTURE 2.3 (Pritchard et al. 2000) was used to estimate the number of genetically distinct subpopulations, assuming the admixture model with correlated allele frequencies.

Although photographic evidence suggests population subdivisions, repeated sightings of killer whales throughout the Aleutian Islands indicate infrequent movement between neighboring geographic strata (National Marine Mammal Laboratory, unpublished data; Durban et al. 2010). In light of these movements and the generally weak signals of population genetic structure resolved for other cetacean populations, it is reasonable to expect relatively weak signals of genetic differentiation. As such, we applied the new models of Hubisz et al. (2009), incorporating general sample locations to inform cluster assignments, rather than the original STRUCTURE model of Pritchard et al. (2000) that incorporates prior information based on the existence of relatively well-supported discrete populations. The sampling location prior (LOCPRIOR) was assigned according to the a priori geographic strata described above. STRUCTURE was run independently both with and without the sampling location prior. We executed 5 independent runs of 10^5 iterations (after burn-in of 10^5 iterations) for each model to estimate the probability support for each number of candidate clusters, k , from 1 to 20. The most likely number of clusters, k , was determined by the method of Pritchard et al. (2000). We also estimated the statistic Δk that quantifies the second-order rate of change in log-likelihood across the range of k values as described by

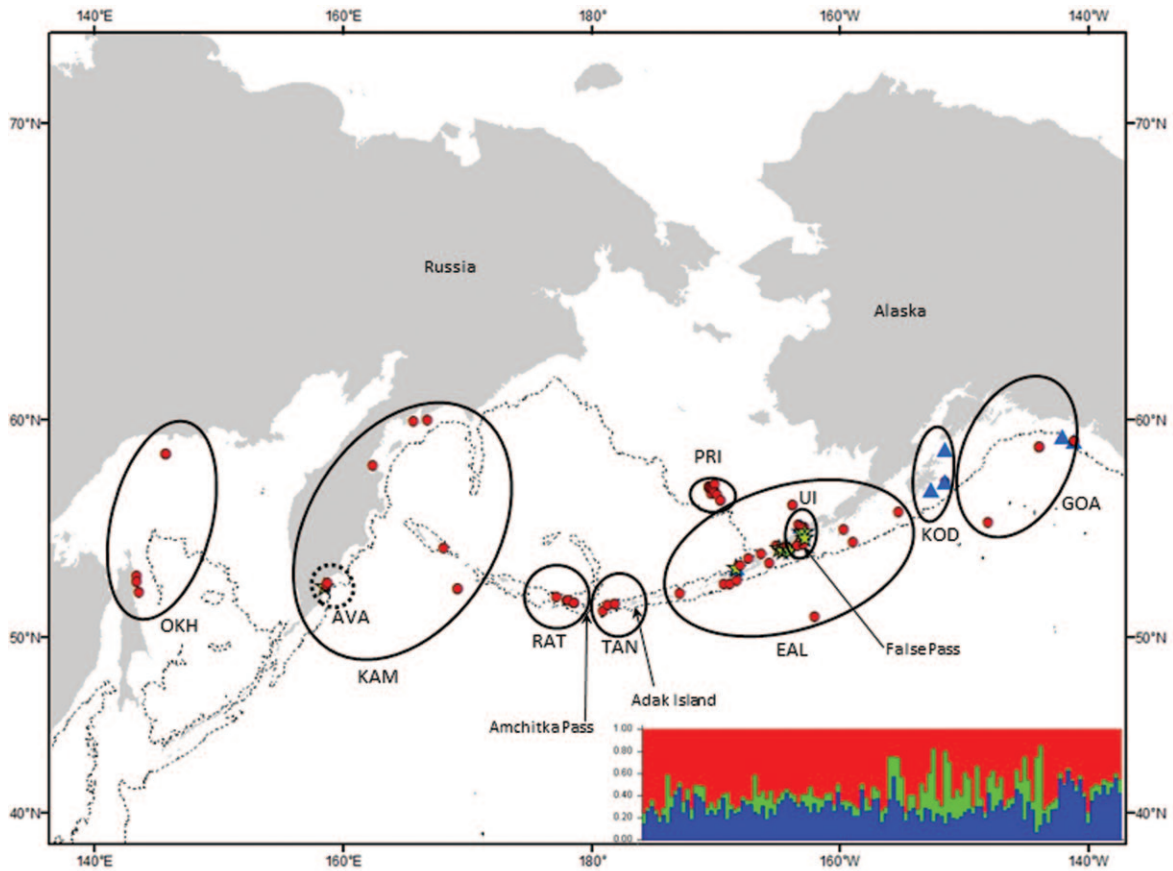


Figure 2. Transient killer whale samples included in this study plotted according to biopsy sample locations. **(a)** Solid line ellipses indicate the extent of a priori geographic strata. Dotted lines surround a putative stratum indicated by Wombling analyses and included in pairwise tests of genetic differentiation. Symbols representing individual samples are colored according to the STRUCTURE cluster (model for $k = 3$) to which they were assigned with the highest probability (mean \pm SD = 0.591 ± 0.100). Inset figure shows the STRUCTURE bar plot ($k = 3$), where each vertical bar represents the proportional membership of individual whales within each of inferred genetic clusters, individuals are ordered by longitude. **(b)** Ellipses indicate a posteriori geographic strata based on analysis of nDNA and mtDNA data. Individual samples are coded according to control region mtDNA haplotype. Inset figure shows regions of genetic discontinuity (light grey) identified by WOMBOSOFT indicating significant putative genetic boundaries for transient killer whales. The 1000 m bathymetric depth contour is indicated by a thin broken line.

Evanno et al. (2005) and directly examined STRUCTURE bar plots for likely values of k .

Genetic cluster analyses were performed for the 2 ecotypes separately, acknowledging the recent findings of mitogenomic analyses that indicated high levels of genetic divergence suggesting that these 2 North Pacific ecotypes may in fact represent separate species (Foote et al. 2011; Morin et al. 2010). In addition to samples collected in the northern North Pacific, STRUCTURE analysis of the resident killer whale data set included a subset of whales ($n = 11$) from the southern resident killer whale (SRKW) population. Despite the relatively continuous distribution of resident killer whales along the west coast of North America, a number of genetically and demographically distinct populations are currently recognized. The SRKW population is recognized as a distinct population segment inhabiting the waters between British Columbia and

northern California and is both geographically segregated and genetically distinct from the Alaskan populations (Barrett-Lennard 2000; Ford et al. 2000; Krahn et al. 2004; Ford et al. 2011). Furthermore, recent genetic analyses found no evidence to suggest that calves were sired by males outside the population, further supporting a lack of gene flow between the SRKW population and neighboring populations (Ford et al. 2011). This subset of SRKW samples was included to provide an independent method for assessing the model's ability to identify this set of samples as a unique genetic cluster.

Quantifying Genetic Differentiation among Subpopulations

Patterns of genetic differentiation among a priori strata were examined for each ecotype using microsatellite genotypes

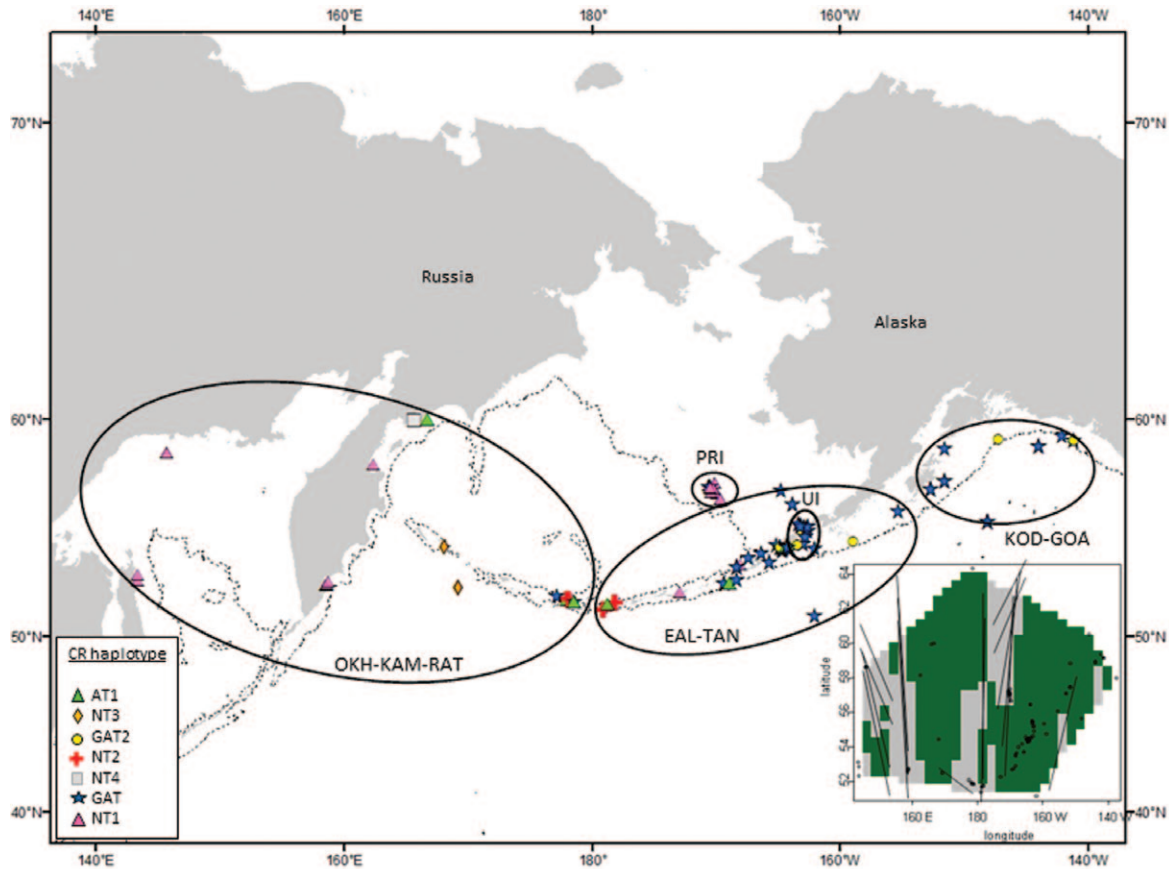


Figure 2. Continued

and mtDNA sequences. Lack of statistical support for geographically neighboring strata was taken as evidence of larger geographic population strata, and putative population boundaries were redrawn accordingly. The spatial extent of genetic clusters inferred from the results of both WOMBOSFT and STRUCTURE were compared with a priori strata. Where the spatial genetic models suggested regions of significant genetic differentiation not reflected by the original a priori subdivisions, new boundaries were drawn a posteriori, and pairwise measures of genetic differentiation among these secondary putative strata were recalculated as described above for both mtDNA and nDNA to provide quantitative metrics for comparison.

Results

Genetic Diversity and Ecotype Differentiation

Molecular genetic analyses were applied to 462 killer whale biopsy samples collected throughout the study range between the northern Gulf of Alaska and the Sea of Okhotsk (Table 1; Figures 1a and 2a; see Supplementary Appendix 2 online). Ecotype was determined for each sample on the basis of photographic (phenotypic) evidence and mtDNA control region haplotype for 98.67% of samples. The absence of

discrepancies between the mtDNA data and photographic-based ecotype assignments clearly supports the validity of these 2 independent methods for ecotype determination and corroborates previous findings for North Pacific killer whales (Durban et al. 2010; Matkin et al. 2007; Zerbini et al. 2007).

Ten unique haplotypes (Table 2) were defined based on nucleotide differences across the mitochondrial control region (~988bp). Both haplotypic and nucleotide diversity were low, consistent with expectations considering previously published studies of killer whale mitochondrial diversity (Table 3; Hoelzel et al. 2002; Zerbini et al. 2007; Morin et al. 2010). Seven mtDNA haplotypes were detected from transient killer whale samples ($n = 153$), whereas only 3 haplotypes were represented among the resident killer whale samples ($n = 288$), with one of these (NEWR) found in only a single whale. No mtDNA haplotypes were shared between the 2 ecotypes. The geographic distribution of the 2 common resident haplotypes was strongly differentiated by a break ($F_{ST} = 0.898$, $P < 0.0001$; $\Phi_{ST} = 0.915$, $P < 0.0001$) at Samalga Pass (170°W), delimiting the western domination by NR and the eastern domination by the SR haplotypes (Figure 1b). Only 5 samples with the NR haplotype were found west of Samalga Pass, but both haplotypes co-occurred in the GOA east of KOD (153°W). In contrast, the distribution of control region haplotypes for transient killer whales was much less

Table 2 Control region (mtDNA) haplotype identity and frequency across sampled resident and transient killer whales for which high quality sequences were generated ($n = 405$)

GenBank accession number	Variable sites	Ecotype	Frequency	Common names
	122234444457			
	267980499938			
	475530934637			
DQ399077	TGTATACACCTA	Resident	176	SR, ENPSR
DQ399078T..	Resident	86	NR, ENPNR
DQ399074	..C.....	Resident	1	NEWR
DQ399082	...GC.T.T.CG	Transient	17	AT1
DQ399081	...CGT.T.CG	Transient	68	GAT
DQ399080GT.T.CG	Transient	11	GAT2, ENPT2
DQ399075	.A..CGT.T.C.	Transient	35	NT1
DQ399076	C...CGT.T.CG	Transient	6	NT2
GU187157	..CGC.T.T.CG	Transient	3	NT3
GU187161	.A..CGTGT.C.	Transient	2	NT4

Variable nucleotide sites within the 980bp mtDNA fragment are indicated.

discrete (Figure 2b) although differences in the frequency of occurrence were evident across the region.

All 27 microsatellite loci were polymorphic. The number of alleles per locus ranged from 3 (Tr04) to 12 (EV37Mn), with an average of 7.22 alleles per locus (see Supplementary Appendix 1 online). Evidence of private alleles was found for both resident and transient ecotypes (Table 3). In general, genetic diversity was higher among transient killer whales (Table 3). The average rate of missing data per locus due to amplification errors was 11.11% (SD = 3.24%), excluding 10 samples that failed to amplify at all loci due to poor sample/DNA quality. Global tests for deviation from HWE within each ecotype revealed heterozygote deficiencies for 7 out of the 27 loci (EV5Pm, KW207, Dde66, 415/416, GATA53, 417/418, and FCB5). However, only KW207 showed evidence of significant departures from HWE for both ecotypes after correction for multiple tests. Plots of H_O/H_E (see Supplementary Appendix 3 online) for each locus confirmed an obvious heterozygote deficit for KW207, and this locus was subsequently dropped from all further analyses. No evidence of genotypic disequilibrium was detected among loci after correction for multiple tests.

Examination of multilocus genotypes for evidence of duplicate genotypes revealed multiple “recaptures” of 23 genotypes, including 21 duplicate and 2 triplicate samples. Original electropherograms were carefully reviewed for all putative matching genotypes mismatching at ≤ 3 loci. A per-allele genotyping error rate of 0.24% was empirically estimated from replicated positive control samples. The most conservative estimate of probability of identity ($P_{(ID)sib}$) was used to provide a lower bound on the number of loci required to reliably distinguish among even closely related individuals. Calculating $P_{(ID)sib}$ for an increasing number of loci, with increasing heterozygosity, indicated that a minimum of 10 loci were required to achieve a conservative $P_{(ID)sib}$ estimate of 0.00078. This probability of identity was used to identify genotypes of sufficient quality, and all samples typed at fewer than 10 loci were removed from subsequent analyses. After

Table 3 Measures of genetic diversity for both nuclear and mitochondrial loci

	Resident killer whales	Transient killer whales
mtDNA		
n	265	142
haplotypes	3	7
h	0.4503 \pm 0.0198	0.6815 \pm 0.0303
π	0.0005 \pm 0.00046	0.0042 \pm 0.0023
Microsatellite		
n	263	143
AR	3.647 (\pm 0.917)	6.701 (\pm 2.242)
N_A	4.000 (\pm 1.095)	6.769 (\pm 2.303)
H_O	0.441 (\pm 0.145)	0.597 (\pm 0.181)
H_E	0.479 (\pm 0.153)	0.647 (\pm 0.184)
F_{IS}	0.113 (\pm 0.190)	0.075 (\pm 0.076)
A_{PRI}	0.577 (\pm 0.138)	3.346 (\pm 0.363)

Values reflect the final data set of 26 microsatellite loci after the removal of duplicate genotypes and poor quality samples that failed across all loci.

h , haplotypic diversity; π nucleotide diversity; AR , allelic richness; N_A , mean number of alleles; A_{PRI} , private alleles averaged across all loci.

the removal of duplicate and triplicate genotypes, and samples typed at ≤ 10 loci, a total of 391 individuals (residents = 264; transients = 127) were included in all spatial genetic analyses.

PCA plots showed clear clustering of samples by ecotype (Figure 3), and 99.8% of samples correctly self-assigned to ecotype using GeneClass. The single sample that misassigned had a probability of assignment of 54% to the alternate population, but assigned to the correct population with a probability of 46%. This assignment ambiguity was likely attributed to missing data at 15 out of 26 loci. All 6 samples of unknown type were assigned to the resident ecotype with an average assignment value of 0.929 (\pm 0.075), supporting the clustering observed in the PCA plot, and were therefore determined to originate from a resident killer whale population. In addition to the absence of shared mtDNA haplotypes

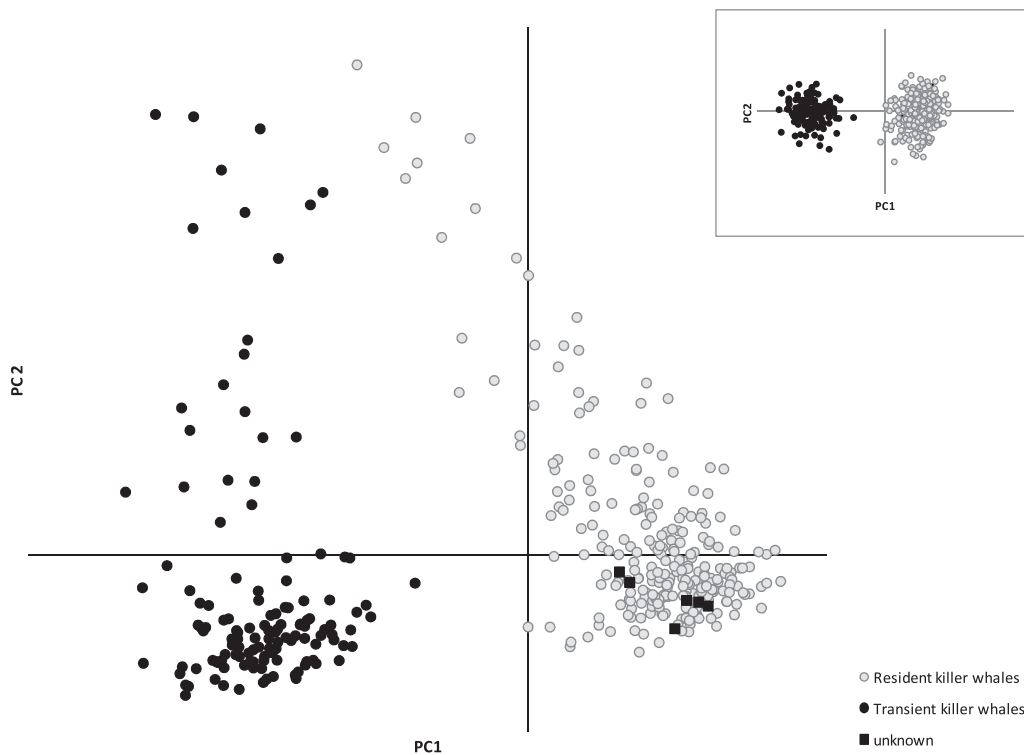


Figure 3. Plot of first 2 principal coordinates based on microsatellite data (26 loci) for all killer whale samples genotyped from the northern North Pacific. The long converging tails are an artifact of samples with incomplete genotypes and are eliminated when the data set is further restricted to samples genotyped at ≥ 20 loci ($n = 372$; inset figure).

(Table 2; $F_{ST} = 0.447$, $P < 0.0001$; $\Phi_{ST} = 0.865$, $P < 0.0001$), estimates of genetic distance indicated highly significant nDNA divergence between the 2 North Pacific killer whale ecotypes ($F_{ST} = 0.2104$, $F'_{ST} = 0.4690$, $P = 0.0001$). The deep genetic divergence between ecotypes was further supported by a cluster analysis performed in STRUCTURE, without prior information on ecotype or sampling location. The results grouped all North Pacific samples into one of two clusters, assigning individual samples with remarkable confidence (mean \pm SD = 0.9950 ± 0.01502). All individual whales correctly assigned to one of two clusters comprised exclusively of either resident or transient killer whales.

Identification of Spatial Genetic Clusters

Relatedness estimates (R_{LR}) based on a simulated data set using the observed allele frequencies resulted in a maximum estimate of R_{LR} for unrelated pairs of individuals (UR_{MAX}) of 0.571 (mean \pm SD = 0.001 ± 0.086) for transient killer whales, and $UR_{MAX} = 0.816$ (mean \pm SD = 0.0007 ± 0.129) for resident killer whales. Using UR_{MAX} as a minimum threshold for estimates of relatedness between potential kin, 9 pairs of resident and 4 pairs of transient killer whales were identified as putative close relatives. One individual from each pair of putative relatives was removed from the data set. Subsequent data analyses were performed on the data representing only unrelated individuals, and all

spatial genetic analyses were conducted separately for each ecotype.

Genetic Structure of Resident Killer Whales

Measures of genetic differentiation among the 5 putative a priori strata of resident killer whales showed significant mtDNA differentiation among all neighboring strata in the Aleutian Islands, and significant nDNA genetic differentiation among all pairwise comparisons except RUS and WAL (Table 4a). In general, measures of genetic divergence between geographically adjacent strata were in agreement across all metrics used, and only chi square failed to support significant subdivision between the 2 geographically adjacent regions represented by CAL and WAL (see Supplementary Appendix 4 online). Pairwise measures of differentiation among a priori strata based on mtDNA sequences also indicated significant genetic differences for 7 out of 10 pairwise comparisons (Table 4a).

The WOMBOSFT analysis indicated the presence of significant genetic boundaries at Buldir Pass between WAL and CAL, and between EAL and GOA, but did not find genetic discontinuity between CAL and EAL (Figure 1b). In the western extent of the study area, putative genetic boundaries were also indicated within the RUS region separating the Kuril Islands (KUR) and Karaginsky Gulf (KAR) from Kamchatka Peninsula (Figure 1b).

Table 4 Pairwise measures of genetic differentiation based on both mtDNA and nDNA among resident killer whales for both (a) a priori and (b) a posteriori geographic strata

(a)	GOA	EAL	CAL	RUS	WAL
GOA	—	0.057	0.824*	0.965*	0.898*
EAL	0.040*	—	0.905*	1.000*	1.000*
CAL	0.063*	0.033*	—	0.124*	−0.004
RUS	0.094*	0.046*	0.033*	—	0.000
WAL	0.085*	0.039*	0.036*	0.009	—
(b)	GOA	EAL-TRI	CAL	WAL-RUS	
GOA	—	0.180*	0.783*	0.962*	
EAL-TRI	0.074*	—	0.915*	1.000*	
CAL	0.114*	0.031*	—	0.131*	
WAL-RUS	0.154*	0.036*	0.029*	—	

Estimates of F'_{ST} (nDNA) are presented below the diagonal and Φ_{ST} (mtDNA) are presented above the diagonal in (a) and (b) for the indicated population strata. Asterisks (*) indicate $P \leq 0.05$ based on 10 000 random permutations of the original data set. A complete list of all F'_{ST} analogs based on nDNA presented in [Supplementary Appendices 4 and 7](#) online.

STRUCTURE indicated the most likely number of subpopulations to be 5 when comparing the values of k (number of clusters) estimated by the methods of Pritchard et al. (2000) and Evanno et al. (2005; see [Supplementary Appendix 5](#) online). As expected, running the model without prior information on sampling location suggested fewer genetic clusters ($k = 3$) with a lower average probability of assignment to the most likely cluster (without LOCPRIOR: mean \pm SD = 0.577 ± 0.123 ; with LOCPRIOR: mean \pm SD = 0.677 ± 0.143) reflecting the positive effect of the location prior on the model's ability to detect weak genetic structure. All STRUCTURE results (both with and without LOCPRIOR) identified the southern resident killer whale samples as a unique genetic cluster providing evidence of the model's ability to accurately identify discontinuous populations (inset, [Figure 1a](#)).

The distribution of genetic clusters based on the results of the STRUCTURE model incorporating the LOCPRIOR supported a population break within the Aleutian Islands between the a priori strata CAL and EAL at Samalga Pass (170°W), as well as a break between EAL and GOA west of Kodiak Island ([Figure 1a](#)). Whales sampled around the Trinity Islands (TRI) were assigned to 3 different genetic clusters. Within CAL, STRUCTURE assigned samples either to a cluster comprised of whales sampled in RUS-WAL ($n = 46$) or to a unique CAL cluster ($n = 48$) with nearly equally probability. No subdivision was indicated in the western regions of the study area within RUS or WAL ([Figure 1a](#)).

To evaluate the additional subdivisions suggested by WOMBOSOF and STRUCTURE, we revised boundaries and recalculated measures of genetic differentiation. RUS was divided into 3 regions (Kuril Islands (KUR), Kamchatka Peninsula (KAM), and Karaginsky Gulf (KAR)) and the Trinity Islands (TRI) separated from the other GOA samples ([Figure 1a](#)). Although WOMBOSOF suggested population subdivisions within the Russian samples, pairwise measures of genetic differentiation failed to support significant divergence between the discontinuous regions of KAR and KUR ($F'_{ST} = 0.029$, $F'_{ST} = 0.054$, $P = 0.120$; see [Supplementary](#)

[Appendix 7](#) online). However, both of these regions were significantly differentiated from the adjacent WAL-KAM (KAR vs. WAL-KAM, $F'_{ST} = 0.027$, $P = 0.007$; KUR vs. WAL-KAM, $F'_{ST} = 0.040$, $P = 0.006$; see [Supplementary Appendix 7](#) online), suggesting subdivision within the westernmost sampled regions. Significant divergence between the whales sampled around the Trinity Islands (TRI) and those in northern GOA ($F'_{ST} = 0.029$, $F'_{ST} = 0.055$, $P = 0.009$), but a lack of differentiation between EAL and TRI ($F'_{ST} = 0.008$, $F'_{ST} = 0.016$, $P = 0.115$) suggested that the genetic boundary for EAL may extend further east than that reflected by the a priori strata.

From these a posteriori analyses, we consider that the data support differentiation among 4 resident killer whale subpopulations (WAL-RUS, CAL, EAL-TRI, and GOA; [Figure 1b](#)). Measures of genetic differentiation among these a posteriori subpopulations supported the genetic divergence among these subpopulations based on both nuclear genotypic data ($F'_{ST} = 0.031$, $F'_{ST} = 0.058$, $P < 0.001$) and mtDNA control region sequences ($F'_{ST} = 0.904$, $P < 0.0001$; $\Phi_{ST} = 0.916$, $P < 0.0001$). Pairwise measures of genetic differentiation based on mtDNA sequence data did not support significant divergence among the a posteriori subdivisions west of Samalga Pass ([Table 4b](#)). This is likely attributable to the extremely low genetic diversity within the mtDNA control region resulting in fixed haplotypes that are shared among populations of piscivorous killer whales.

Genetic Structure of Transient Killer Whales

Pairwise measures of genetic differentiation among the 9 a priori strata of transient killer whales shared no significant mtDNA divergence (Φ_{ST}) among all strata east of Adak Island, except for PRI ([Figure 2a](#); [Table 5a](#)). Transients sampled around the Pribilof Islands (PRI) were also significantly differentiated from all strata east of Kamchatka Peninsula ([Table 4a](#)). There was no significant mtDNA differentiation between both TAN-RAT and TAN-KAM ([Table 5a](#)).

Table 5 Pairwise measures of genetic differentiation based on both mtDNA and nDNA among transient killer whales for both (a) a priori and (b) a posteriori geographic strata

(a)								
GOA	KOD	EAL	UI	PRI	TAN	RAT	KAM	OKH
—	−0.010	−0.053	−0.045	0.574*	0.280*	0.257*	0.316*	0.574*
0.064	—	−0.027	0.040	0.594*	0.399*	0.346*	0.311*	0.661*
0.052*	0.061*	—	0.013	0.502*	0.272*	0.250*	0.341*	0.514*
0.034	0.080*	0.041*	—	0.624*	0.487*	0.436*	0.430*	0.698*
0.053*	0.033	0.019*	0.059*	—	0.632*	0.624*	0.179*	−0.020
0.034	0.125*	0.021	0.016	0.066*	—	−0.056	0.248	0.503*
0.009	0.086*	0.041*	0.076*	0.034*	0.067*	—	0.270*	0.518*
−0.007	−0.015	0.023	0.044*	0.013	0.041	−0.007	—	−0.015
0.060	0.129*	0.032	0.108*	0.098*	0.072*	0.022	0.049	—
(b)								
KOD-GOA	EAL-TAN	UI	PRIB	OKH-KAM-RAT				
—	−0.007	−0.033	0.605*	0.222*				
0.041*	—	0.036	0.484*	0.158*				
0.032	0.034	—	0.624*	0.259*				
0.029*	0.024*	0.059*	—	0.212*				
0.011	0.031*	0.065*	0.035*	—				

Estimates of F'_{ST} (nDNA) are presented below the diagonal and Φ_{ST} (mtDNA) are presented above the diagonal in (a) and (b) for the indicated population strata. Asterisks (*) indicate $P \leq 0.05$ based on 1 000 random permutations of the original data set. A complete list of all F'_{ST} analogs based on nDNA presented in [Supplementary Appendices 4 and 7](#) online.

Estimates of differentiation based on nuclear microsatellite data revealed little or no significant genetic differentiation among some geographically adjacent a priori strata, suggesting larger subpopulations than the original strata tested ([Table 5a](#); see [Supplementary Appendix 4](#) online). Lack of significant differentiation among whales sampled west of Amchitka Pass (OKH, KAM, and RAT) provided strong evidence for a point of geographic subdivision at Amchitka Pass (179°E). Results also indicated a lack of genetic differentiation east of Kodiak Island (KOD and GOA). In the eastern Aleutians, significant nDNA differentiation was indicated between EAL and neighboring PRI to the north, but there was a lack of statistical support for the a priori split between EAL and TAN, to the west ([Table 5a](#)). Interestingly, significant genetic differentiation was apparent when comparing whales observed in spring assemblages around Unimak Island (UI) to the seasonally sympatric whales sampled in the EAL stratum ([Table 5a](#)). In general, all measures of genetic divergence between geographically adjacent strata concurred, with the exception of chi square which was marginally nonsignificant, failing to support the putative subdivision between RAT and TAN (see [Supplementary Appendix 4](#) online).

WOMBOSOFT analysis supported the broad patterns indicated above, highlighting both Amchitka Pass (179°E) as a significant genetic boundary between the western Aleutians (RAT) and the central Aleutians (TAN), and a zone of genetic differentiation within the Pribilof Islands (inset, [Figure 2b](#)). In Russian waters, the WOMBOSOFT analyses suggested a latitudinal division across Kamchatka Peninsula (KAM) in the region of Avacha Bay (53°N) (inset, [Figure 2b](#)).

STRUCTURE without prior information on sampling location provided little evidence of genetic structure with all

individuals being assigned to one of two clusters with nearly equal probability (mean \pm SD = 0.546 \pm 0.028). However, when location information (LOCPRIOR) was included, log-likelihood values suggest that transients in the sampled area most likely represent 3 genetic clusters (see [Supplementary Appendix 6](#) online). While the evidence of genetic structure was weak, probabilistic cluster assignments for individual whales differentiated a small number of GOA samples ($n = 9$) and a subset of EAL samples ($n = 10$) around Unimak Island from all others ([Figure 2a](#)). Eight out of 10 of the individual whales assigned to the cluster around Unimak Island originated from the UI a priori stratum.

Lack of genetic differentiation among some a priori strata, as well as results from both WOMBOSOFT and STRUCTURE, generally indicated fewer, larger population subdivisions than the 9 originally postulated ([Table 5a](#)). To reflect these results, regional population strata were redrawn into 5 larger a posteriori strata as follows: all samples west of Amchitka Pass (179°) were grouped together (OKH-KAM-RAT), samples from the central Aleutians (TAN) were grouped with those from the eastern Aleutians (EAL), and all samples from the Gulf of Alaska (KOD-GOA) were grouped into a single stratum ([Figure 2b](#)). Substructuring within the samples collected along the Kamchatka peninsula (KAM) was examined by comparing whales sampled within Avacha Gulf (AVA) to all others in KAM to further examine the zone of genetic discontinuity indicated by WOMBOSOFT analyses.

Revised estimates of genetic differentiation (OKH-KAM-RAT, EAL-TAN, PRI, UI, and KOD-GOA; [Figure 2b](#)) supported the 5 a posteriori strata for both nuclear genotypes ($F'_{ST} = 0.012$, $F'_{ST} = 0.034$, $P = 0.0009$; [Table 5b](#)) and mtDNA control region sequences ($F'_{ST} = 0.271$, $P < 0.0001$;

$\Phi_{ST} = 0.295$, $P < 0.0001$; Table 5b). Genetic differentiation among the Russian regions, including Avacha Gulf (AVA), were not significant ($F_{ST} = 0.012$, $P = 0.183$), most likely reflecting a lack of power due to extremely small sample sizes in this region for transient whales at the current time (AVA, $n = 4$).

Discussion

Using a suite of 26 microsatellite loci and a large number of georeferenced samples, we have provided the most comprehensive study of killer whale population genetic structure in the North Pacific to date. Analysis of molecular genetic data revealed significant levels of population genetic subdivision within the 2 predominant ecotypes of the genus *Orcinus* across the northern North Pacific using both mitochondrial control region sequences and nuclear microsatellite genotypes. Strong evidence of genetic divergence among neighboring geographic regions indicated multiple populations within the currently recognized stocks for both resident and transient killer whales. However, patterns of population genetic subdivision suggested some notable differences in the geographic structuring of populations between the 2 ecotypes.

Genetic Divergence among Ecotypes

Estimates of genetic distance between the 2 predominant North Pacific ecotypes indicate negligible levels of gene flow between ecotypes, confirming the findings of previous studies of ecotypic variation, and highlighting the genetic and demographic isolation of these 2 divergent evolutionary lineages in the North Pacific (Hoelzel and Dover 1991; Hoelzel et al. 2007; Morin et al. 2010; Pilot et al. 2010). This study more than doubled the total number of killer whale samples representing Alaska and Russia compared with previous studies (Hoelzel et al. 2007; Pilot et al. 2010) and substantially increased the number of polymorphic microsatellites from 16 to 26 loci. Recently, analysis of mitogenome sequences demonstrate phylogenetic sorting of ecotypes and suggest that transient killer whales should be elevated to full species status (Morin et al. 2010). The lack of shared mtDNA haplotypes and the significant genetic differentiation of nDNA data in this study support these findings and highlight the contemporary genetic divergence of the 2 ecotypes.

Geographic Structure of North Pacific Resident Killer Whales

Our analyses of the resident killer whale data set supported the existence of 4 longitudinally divided subpopulations across the North Pacific and Bering Sea. The eastern Aleutians subpopulation appears to diverge from the northern Gulf of Alaska in the waters around Kodiak Island. The 2 other major points of population subdivision coincide with 2 major island passes: Samalga Pass and Buldir Pass. The presence of population subdivision at Samalga Pass indicated by Bayesian cluster analysis of nDNA genotypic data was supported by a striking shift in the frequency of mtDNA haplotypes and also supported by all pairwise measures of

genetic differentiation examined for resident killer whales. Samalga Pass has previously been recognized as a physical and biogeographic boundary between the eastern and central Aleutians (Ladd et al. 2005). WOMBOSoft analyses also indicated the presence of 2 possible genetic boundaries within Russia. Pairwise measures of genetic divergence supported genetic discontinuity between Kamchatka Peninsula and the Kuril Islands; however, there was a lack of evidence of genetic differentiation between the 2 noncontiguous regions separated by KAM (KAR and KUR), which may be attributable to small sample sizes (7 and 6, respectively). These major geographic subdivisions within the resident killer whale ecotype are consistent both with direct evidence of individual movements and with the geographic extent of social networks (Fearnbach H et al., unpublished data) and are supported by broad regional differences in both stable isotopes and persistent organic pollutants suggesting that differences in prey across the northern North Pacific may be a driving factor shaping population subdivisions (Krahn et al. 2007).

According to nDNA data, the point of subdivision between resident killer whales in the northern Gulf of Alaska (GOA) and the eastern Aleutians is in the region of Kodiak Island. Despite the indication of a genetic boundary west of the Trinity Islands, pairwise comparisons among strata suggest that whales sampled in this region (TRI) were significantly differentiated from GOA and most likely continuous with the eastern Aleutians subpopulation. Direct observations of photographically documented killer whales indicate a single population in the northern GOA spanning the waters from southeastern Alaska to Kodiak Island (Matkin 1997; Matkin et al. 1999), which is socially and spatially distinct from whales further west (Fearnbach 2012; Matkin et al. 2007). Association data and acoustic analyses also support an eastern Aleutian population of resident killer whales that interacts infrequently with Gulf of Alaska animals (Fearnbach 2012; Matkin et al. 2007). However, recently acquired data from satellite transmitter tags highlight marked seasonal differences in the movement patterns of whales in the northern and eastern Gulf of Alaska, as well as differences in core areas among matriline (Matkin CO et al., unpublished data). These data emphasize the extreme mobility of these animals and underscore the limitations of inferring fixed boundaries from instantaneous samples.

Geographic Structure of North Pacific Transient Killer Whales

In contrast to the longitudinally defined geographic subpopulations of the resident killer whales, population genetic boundaries for transient killer whales indicate a few large geographic subdivisions, interspersed with smaller neighboring or seasonally sympatric subpopulations. As with the resident killer whales, genotypic data indicate that the waters around Kodiak Island likely represent the easternmost point of subdivision between EAL and GOA. Direct data on the movements of transient killer whales also support population differences between the eastern Aleutians and the Gulf

of Alaska (Matkin et al. 2007; Durban et al. 2010; Matkin et al. 2012). The westernmost subpopulation extends further east than that resolved from the resident genotypic data, encompassing both Russian areas (OKH and KAM) and those of the Rat Islands in the Aleutians, extending as far east as Amchitka Pass (179°W; Figure 2b). Pairwise measures of genetic differentiation indicated significant divergence between the neighboring a priori strata of Tanaga and Rat Island groups, supporting this as a significant place of genetic subdivision between the central and western Aleutians. It is important to note, however, that limited sample sizes in the western reaches of the study area restrict the resolution of population genetic structure west of Amchitka Pass and additional samples would greatly enhance our ability to determine contemporary levels of gene flow among the western Aleutians and Commander Islands.

Within the eastern/central Aleutians, our analyses provided strong evidence for multiple populations with a seasonal co-occurrence. Nuclear microsatellite data suggest the presence of 1 larger population cluster extending from the western GOA to Amchitka Pass, as well as a smaller sympatric subpopulation around Unimak Island. Observations of transients around Unimak Island in spring have revealed aggregations of killer whales that are distinct in acoustic call repertoire, patterns of association, and timing of occurrence compared with those further west (Matkin et al. 2007; Durban et al. 2010; Barrett-Lennard et al. 2011). During May and early June, concentrations of transient killer whales have been observed intercepting and preying on northward-migrating gray whales in the waters around Unimak Island (Barrett-Lennard et al. 2011). Genotypic data in this study were found to support the a priori hypothesis that whales observed in these spring foraging assemblages around Unimak Island are significantly divergent from some conspecifics sampled in the summer months in the adjacent eastern Aleutians. These signals of fine-scale sympatric genetic clusters may reflect social or ecological specializations occurring on a relatively small scale, or temporary/seasonal sympatry of killer whale populations during the summer months.

Unlike the fish-eating ecotype, the EAL subpopulation of transient killer whales was found to be genetically distinct from those around the adjacent Pribilof Islands in the Bering Sea based on both nDNA and mtDNA. A small number of photographically documented movements between EAL/UI and PRI as well as ongoing social network analyses support the existence of 2 neighboring strata that are connected by infrequent movements of individual whales (Wade P and Durban J, unpublished data). Killer whales are physically capable of undertaking extensive movements (Durban and Pitman 2011), likely responding to changes in prey availability, social requirements, or physiological constraints. Although satellite telemetry data and direct observations have demonstrated the capability of long-range movements by transient killer whales (Goley and Straley 1994; Matkin et al. 2012), individual resightings in our study region suggest seasonally based site fidelity with an average maximum straight line distance of only 95 km (minimum 2 km and maximum 507 km; Durban J, unpublished data) between repeated sightings

across consecutive years. This indicates that although individual whales may not remain year-round in a given area, they are predictable in returning to seasonal prey aggregations (Durban et al. 2010). Seasonal changes in the abundance and distribution of key prey species may affect the degree of geographical overlap of neighboring subpopulations resulting from short-term convergence on prey aggregations.

Factors Shaping the Structuring of Killer Whale Populations

Marked seasonal variability in prey availability has been linked to temporal movements of transient killer whales in the North Pacific, often coinciding with seasonal concentrations of prey (Baird and Dill 1995; Matkin et al. 2002; Matkin et al. 2007; Dahlheim et al. 2009; Barrett-Lennard et al. 2011). For example, peak abundance in transient killer whale sightings at the Chiswell Island Steller sea lion rookery (Kenai Fjords) coincided with the peak in pinniped abundance (Maniscalco et al. 2007), and killer whale sightings around Unimak Island declined rapidly at the end of May following the migration of the majority of gray whale females and young-of-the-year calves (Barrett-Lennard et al. 2011). Stable isotope analyses further support observational data suggesting seasonal changes in the primary prey consumed by transient killer whales (Krahn et al. 2007). Partial sympatry in killer whale populations has also been described in the North Atlantic where population structuring appears to be largely driven by prey specialization (Foote et al. 2009, 2011). Among piscivorous killer whales in the eastern North Atlantic, potential geographic contact zones have been identified based on data from seasonal prey movements (Foote et al. 2011). Such temporal and spatial convergence of mobile predators not only provides incidental opportunities for male-mediated gene flow but also provides unique opportunities for ecological specialization.

Both killer whale ecotypes exhibited a lack of genetic differentiation between the northern and southern sides of the Aleutian Islands on the continental shelf. Despite the defining ecological differences inherent to the 2 killer whale ecotypes, both represent apex predators within the marine ecosystem, and factors such as prey preferences and distribution of preferred prey are likely responsible for shaping geographical population subdivisions. Regional dietary differences characterized for populations of other North Pacific marine mammals reflect similar geographic patterns to the genetic seascape described here for killer whales. Both humpback whales (*Megaptera novaeangliae*) and Steller sea lions foraging at a similar trophic level to resident killer whales exhibit regional differences in diet across the northern North Pacific that are largely correlated with longitude (Sinclair and Zeppelin 2002; Sinclair et al. 2005; Witteveen et al. 2009). A study of humpback whales using stable isotope ratios to infer regional differences among summer feeding grounds indicated a significant break in the western GOA representing a longitudinal shift in prey preferences from fish in the northern GOA to zooplankton in western GOA (Witteveen et al. 2009). That study also revealed dietary differences between

the eastern Aleutians and regions to the west (including the central and western Aleutians and the Commander Islands). Similarly, resident killer whales in Alaska also exhibited an east-to-west gradient in carbon and nitrogen isotope ratios between the GOA and the central Aleutian Islands suggesting regional prey differences (Krahn et al. 2007). Steller sea lions also exhibit marked regional differences in both population trends and prey preferences. Studies of Steller sea lion dietary differences among Aleutian Island rookeries found that diets east of Samalga Pass were more diverse and dominated by walleye pollock (*Theragra chalcogramma*) and salmon (*Oncorhynchus* spp.), compared with diets west of Samalga Pass that were heavily dominated by Atka mackerel (*Pleurogrammus monopterygius*) (Sinclair and Zeppelin 2002).

This longitudinal point of division also separates regions experiencing contrasting population trends within the endangered western stock of Steller sea lions (York et al. 1996; Sinclair and Zeppelin 2002; Call and Loughlin 2005). The identified geographic zone of differentiation among regions located at Samalga Pass corresponds with the geographic break described in this study for resident killer whales (supported by both mtDNA and nDNA data), which are likely feeding at the same trophic level as Steller sea lions and on some of the same prey. Resident killer whales have been observed feeding on salmon in the eastern Aleutians and on Atka mackerel in the central Aleutians (Wade P, Durban J, unpublished data). Both seabird (Jahncke et al. 2005) and zooplankton (Coyle 2005) species distributions also divide at Samalga Pass and it is thought that this area forms a key physical and biogeographic transition zone between the more coastal (or shelf-dominated) ecosystems of the eastern Aleutians and the more oceanic ecosystems of the central Aleutians (Ladd et al. 2005).

Interestingly, the observed patterns of geographic structuring described in this study for transient killer whales failed to support a significant subdivision between the eastern and central Aleutians around Samalga Pass. As apex predators, these killer whales are one step further removed from the direct effects of bottom-up structuring, described above. Although the tertiary consumers on which they prey may exhibit regional differences in population demographics and prey specializations, it is plausible that such effects become increasingly diluted at the top of the food chain, and other factors such as seasonal prey preferences and culturally transmitted prey specializations may assume significant roles in population structuring.

Management Implications

The patterns of genetic structure presented in this study provide strong evidence for the existence of multiple subpopulations of killer whales across the northern North Pacific, highlighting the need to revisit current stock designations. Killer whales in the northern North Pacific are impacted through both direct and indirect interactions with commercial fisheries. Evidence of population differentiation in this highly mobile species is a critical

component for evaluating the impacts of incidental bycatch and estimating predator–prey relationships. A revision of the stock structure could have management implications for fisheries bycatch of resident killer whales in Alaska. Similarly, the geographic subdivision of transient killer whale populations may have implications for interpreting the role of killer whale predation in the decline and lack of recovery of Steller sea lions. However, these data also emphasize the need for additional individual-based data to inform fine-scale genetic analyses in areas such as Unimak Island and the Gulf of Alaska where multiple genetic clusters were indicated. Future individual-based analyses integrating direct observations and genetic data are necessary to resolve the temporal and spatial aspects of genetic structuring, and further our understanding of the localized role of killer whales as top predators and competitors in North Pacific ecosystems.

Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

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