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SNP GENOTYPING AND APPLICATIONS

Sperm whale population structure in the eastern and central North Pacific inferred by the use of single-nucleotide polymorphisms, microsatellites and mitochondrial DNA

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Abstract

We use mitochondrial DNA (mtDNA) (400 bp), six microsatellites and 36 single-nucleotide polymorphisms (SNPs), 20 of which were linked, to investigate population structure of sperm whales (*Physeter macrocephalus*) in the eastern and central North Pacific. SNP markers, reproducible across technologies and laboratories, are ideal for long-term studies of globally distributed species such as sperm whales, a species of conservation concern because of both historical and contemporary impacts. We estimate genetic differentiation among three strata in the temperate to tropical waters where females are found: California Current, Hawai`i and the eastern tropical Pacific. We then consider how males on sub-Arctic foraging grounds assign to these strata. The California Current stratum was differentiated from both the other strata ($P < 0.05$) for mtDNA, microsatellites and SNPs, suggesting that the region supports a demographically independent population and providing the first indication that males may exhibit reproductive philopatry. Comparisons between the Hawai`i stratum and the eastern tropical Pacific stratum are not conclusive at this time. Comparisons with Alaska males were statistically significant, or nearly so, from all three strata and individuals showed mixed assignment to, and few exclusions from, the three potential source strata, suggesting widespread origin of males on sub-Arctic feeding grounds. We show that SNPs have sufficient power to detect population structure even when genetic differentiation is low. There is a need for better analytical methods for SNPs, especially when linked SNPs are used, but SNPs appear to be a valuable marker for long-term studies of globally dispersed and highly mobile species.

Keywords: conservation, *Physeter macrocephalus*, population structure, single-nucleotide polymorphism, sperm whale

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Introduction

Molecular markers are one of our best tools for investigating population structure in globally dispersed and highly mobile marine mammal species. However, the vast and often remote distribution of such species means that sample accumulation is typically slow and laboratory analyses are therefore likely to extend across many years and often many laboratories. The long time span of such studies poses a problem for projects employing

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microsatellite markers. Although the sequencing of mitochondrial DNA (mtDNA) has remained relatively stable over the past two decades, the method of genotyping microsatellites (electrophoresis with allele size inference) makes allele-length estimates dependent on technologies that change over time. These evolving technologies not only impact studies carried out over time in a single laboratory but also impact potential collaborations among laboratories where different technologies may be employed. Single-nucleotide polymorphisms (SNPs), on the other hand, represent a more stable nuclear marker type for use when data sets need to be combined across time and technologies. SNP genotypes are less technology dependent because they represent sequence differences (G, A, T, C), not estimated allele sizes. SNPs also provide the ability to genotype degraded and poor-quality samples (Morin & McCarthy 2007), enabling data sets to be expanded through the use of historical samples. In addition, highly multiplexed pre-amplification of SNP loci prior to genotyping (Morin & McCarthy 2007) has reduced the need to repeatedly use genomic DNA from extremely limited samples, thus preserving these valuable samples for future studies and new technologies. Although the genetic power of individual SNPs is lower than that of individual microsatellites because they are bi-allelic (Chakraborty *et al.* 1999; Krawczak 1999), their high abundance in most genomes, highly efficient and accurate genotyping methods, and sufficient statistical power with a two- to fourfold increase in the number of markers and the use of linked loci are advantages that make them a good marker choice (Kuhner *et al.* 2000; Turakulov & Easteal 2003; Aitken *et al.* 2004; Morin *et al.* 2004, 2007, 2009c; Seddon *et al.* 2005; Anderson & Garza 2006; Morin & McCarthy 2007; Smith & Seeb 2008). Lastly, a lower mutation rate and simple mutation model enable the use of traditional divergence-based statistics such as F_{ST} without concern for impacts of within-group polymorphism or incorrect mutation model (Meirmans & Hedrick 2010).

Sperm whales (*Physeter macropcephalus*) are an example of a species of conservation concern where the slow accumulation of samples means that laboratory analyses have extended over decades. Sperm whales are a continuously distributed species, found in all deep oceans of the world from the equator to the edge of the pack ice and are well known for long-distance movements (Rice 1998). Discovery marks (metal tags shot into the whales during the industrial whaling period and recovered from carcasses during processing) show extensive movements of males and females both north to south and east to west within the North Pacific (Kasuya & Miyashita 1988). Recently, two adult males satellite-tagged off Southeast Alaska moved rapidly through the waters along the west coast of North America, one travelling into the Gulf of California and the other along the coast of southern Mexico, linking these tropical areas with females to Alaskan feeding grounds (Andrews *et al.* 2010; Straley, J.M., Andrews, R.D., Schorr, G.S., Thode, A.M., Calambokidis, J., Lunsford, C.R., Chenoweth, E., and O'Connell, T. in preparation). In the eastern tropical Pacific, photographic matches of individually identifiable flukes have been made between females and immatures sighted in the Gulf of California, the Galapagos Islands, Gulf of Panama, Ecuador, Peru and northern Chile (Whitehead *et al.* 2008). Such extensive movements, and the lack of hiatuses in distribution, make defining feasible study areas difficult. Furthermore, long dive times (typically 30–45 min) (Whitehead 2003) and the large size and remoteness of most of their habitat mean that the accumulation of sperm whale samples is slow. Samples for this study, for example, have been collected over a 35-year period and analysed over a 15-year period. The slow accrual of samples and the global distribution of this species makes it an ideal case for the use of SNPs (Morin *et al.* 2004) and resulted in this species being chosen as the first among marine mammals for which SNPs were developed (Morin *et al.* 2007).

Understanding population structure of sperm whales in the eastern North Pacific has been the subject of intense discussion since the 1970s but little progress has been made (Donovan 1991; Dufault *et al.* 1999). Early efforts to define stocks were often based on patterns of historic whaling activity and catch reports rather than on biological evidence (Donovan 1991). More recently, various types of data including acoustic dialects, association data from photographs, mark–recapture data and morphology all suggest philopatry among female sperm whales (Dufault *et al.* 1999; Whitehead *et al.* 2008). Genetic studies have found low but significant levels of genetic differentiation among ocean basins for mtDNA ($G_{ST} = 0.030$, $P < 0.001$) (Lyrholm & Gyllensten 1998) while studies using microsatellites have found either no significant (Lyrholm *et al.* 1999) or low, but significant differentiation ($\theta = 0.002$, $P < 0.001$) (Bond 1999). Within ocean basins, little evidence of genetic differentiation has been found among areas with females (few statistically significant pairwise comparisons in either the Atlantic or the Pacific), with the exception of isolated basins such as the Mediterranean and Gulf of Mexico (Dillon 1996; Lyrholm & Gyllensten 1998; Bond 1999; Engelhaupt *et al.* 2009).

The unusual social structure of sperm whales further complicates investigations into their population structure. Adult males and females are sexually segregated. Females and their dependent young travel in groups and generally inhabit tropical and temperate waters (Best 1979; Whitehead 2003). These groups are comprised of temporary associations of partially matrilineal (Richard

et al. 1996a, Mesnick *et al.* 2003) and largely stable units (Whitehead *et al.* 1991) which, in turn, are structured into acoustic clans (Weilgart & Whitehead 1997). Maturing males leave the female groups and begin a gradual movement into higher latitudes (Best 1979; Whitehead 2003). The distribution of males extends into sub-Arctic foraging grounds (Rice 1998) from which mature males make periodic movements into warmer waters on an unknown schedule to mate (Whitehead & Arnbom 1987). It is unknown whether males return to their natal grounds to mate and/or roam widely, perhaps even between ocean basins (Rendell *et al.* 2005).

Sperm whales are listed globally by the IUCN (2010) as vulnerable and are protected by International Whaling Commission treaty and, in the United States, by the Endangered Species Act and Marine Mammal Protection Act (MMPA). In the last 200 years, hundreds of thousands of sperm whales were killed in both the Yankee whaling era and the industrial era, which ended in the 1980s (Rice 1998). Efforts to assess the impact of historical whaling, the role of sperm whales in pelagic ecosystems and the current conservation status of stocks are compromised by the absence of a good model of sperm whale population structure. Accidental death in fisheries is one contemporary threat to sperm whales. In Alaska, male sperm whales remove fish from longline gear (depredation) set primarily for sablefish (*Anoplopoma fimbria*) and have been at risk of entanglement from this activity since at least 1997 (Hill *et al.* 1999; Thode *et al.* 2007; Sigler *et al.* 2008; SEASWAP). Fishery interactions with sperm whales in Alaska are regulated under the MMPA. Under this Act, stocks are defined as demographically independent populations for which 'population dynamics … is more a consequence of births and deaths within the group (internal dynamics) rather than immigration and emigration (external dynamics)' (Wade & Angliss 1997). As Martien *et al.* (2010) discuss, this definition is consistent with that of Management Units as proposed by Palsboll *et al.* (2007) and falls into the 'ecological paradigm' of population definitions described by Waples & Gaggiotti (2006). Under this paradigm, detecting population structure through genetic analysis can be quite challenging, as the expected level of differentiation is very low (see Box 1) (Martien *et al.* 2010).

There are currently three stocks of sperm whales recognized in the eastern and central North Pacific for purposes of implementing the MMPA: California/Oregon/Washington, Hawai`i, and North Pacific (Alaska) stocks (Allen & Angliss 2010; Carretta *et al.* 2010). The Alaska stock is unusual among marine mammal stocks in being comprised primarily of adult males and, unlike other stocks, is not self-sustaining, because nearly all births occur elsewhere and few adult females visit the region. Assessing the impact of any possible fishery inter-

Box 1. Estimation of expected levels of genetic differentiation for North Pacific sperm whales

We use the approach outlined by (Palsboll *et al.* 2007) in which results are interpreted relative to the critical level of dispersal and differentiation necessary to meet conservation objectives. Taylor (1997) has shown that, for cetacean species, even populations exchanging dispersers at rates as high as 1% per year will likely require management as separate stocks if the conservation goals of the MMPA are to be met. Given the estimated 31.9 year generation time for sperm whales (Taylor *et al.* 2007), this corresponds to a per-generation dispersal rate (m) of 0.319. The expected level of genetic differentiation between two populations can be calculated using Wright's (1965) formulae:

$$F_{ST}(\text{mtDNA}) = 1/(2N_e m + 1)$$

$$F_{ST}(\text{nDNA}) = 1/(4N_e m + 1)$$

where N_e is the effective population size. The strata with the lowest plausible abundance is Hawai`i with about 7000 whales and the largest plausible abundance is the entire area of the eastern tropical Pacific with up to 23 000 whales (Wade & Gerrodette 1993). For long-lived species, Ne is approximately half the number of breeding adults (Nunney 1993). Assuming a 50:50 sex ratio and 56% mature (Taylor *et al.* 2007), this results in N_e/N of 0.28 for mtDNA and 0.56 for nuclear DNA (nDNA). Therefore, the expected F_{ST} value for sperm whale populations exchanging migrants at a rate of 1% per year (31.9% per generation) ranges from 0.0005 to 0.0016 for mtDNA and from 0.0001 to 0.0004 for nDNA, assuming trivial mutation rates (Balloux *et al.* 2000) (Fig. 1). For this relatively high rate of gene flow the expected level of genetic differentiation between two populations (the effect size) is less than an F_{ST} value of 0.002 over the entire range of plausible abundances. Decreasing the annual dispersal rate to 1/10%, which is certainly demographically trivial, still means that the maximum level of genetic differentiation with the demographic assumptions above is F_{ST} = 0.0157 which differs little from the value for panmixia (F_{ST} = 0). Therefore, positive results for population structure should warrant management as demographically independent populations even if the levels of differentiation are small.

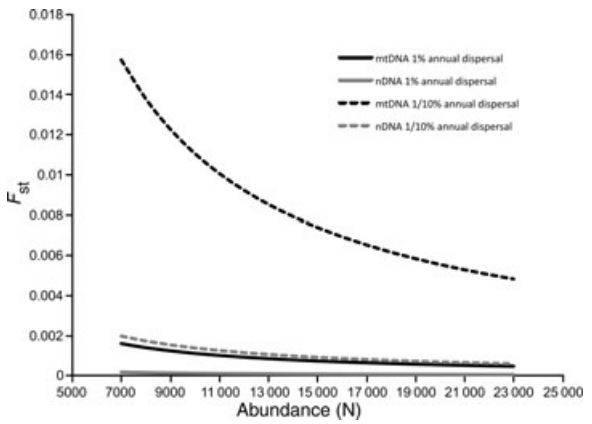

Fig. 1 F_{ST} for mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) for annual dispersal rates of 1% and 1/10% over the range of abundances plausible for sperm whales.

actions on Alaska males should be connected with the abundance of the stock that includes the females with which they reproduce.

In this study, we use mtDNA, microsatellites and SNPs to improve understanding of population structure in the eastern and central North Pacific. Our primary objective is to examine the demographic independence of three strata from temperate and tropical waters where females are found and to assess the relationship of adult males in Alaska to these strata (Fig. 2). We also examine patterns of genetic assignment of predominantly female groups located in waters outside these designated strata (Fig. 2) to give insight into areas where sampling remains poor. As considered in more detail in Box 1, we consider a 1%/year dispersal rate between strata sufficiently low to constitute demographic independence. Owing to the large population sizes, we expect genetic differentiation to be small between strata (F_{ST} <0.0016 for mtDNA and 0.0004 for nDNA; Box 1).

We use tests of genetic differentiation and assignment to address the following questions:

1 Is there genetic differentiation among the three strata with females and young using mtDNA?
2 Is there genetic differentiation among the three strata with females and young using nDNA?
3 Do Alaska males originate from a single stratum?
4 If Alaska males do not originate from a single stratum, how do individuals assign to the three strata examined here?

If males rove among regions but females stay within the region of their birth, then pairwise comparisons between strata using mtDNA should show significant differentiation but pairwise comparisons between strata using nDNA would not. If significant differentiation is found for both mtDNA and nDNA, then both females and males show regional structure. If males in Alaska do not originate from a single stratum, then males mix on their feeding grounds (if answer to question 3 is yes), but may return to their region of birth to mate.

While SNP genotyping produces stable results, analysis of linked SNPs requires considerable effort, and analytical methods for linked markers are still being developed. Therefore, our study also examines the effect of different assumptions that could be made in analysing SNPs on the conclusions about population structure. The compounding complexity of considering uncertainties contributed by sperm whale biology, new statistical measures of population differentiation, multiple markers and various treatment of linked SNP loci results in a large number of data sets and results to report. Fortunately, the different analyses necessary to thoroughly address these uncertainties followed consistent patterns. Here, we present the strata and statistics that we feel best represent sperm whale population structure in the eastern and central North Pacific but invite the reader to fully explore the results of all the analyses in the Supporting information.

Materials and methods

Sample collection

The sample set consisted of 287 tissue samples obtained between 1972 and 2007 (Table S1, Supporting informa-

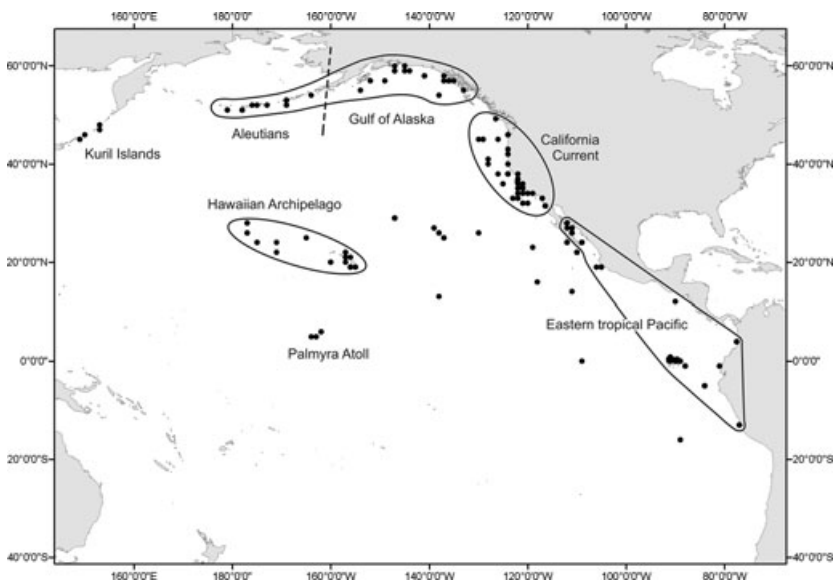

Fig. 2 Map showing sample and strata locations. See text for discussion of strata designations.

tion). Samples were collected from solitary or groups of free-ranging sperm whales by directed biopsy or the collection of sloughed skin during cetacean research surveys (n = 255). Samples were also collected from dead animals stranded on beaches (n = 29), floating dead at sea (n = 1) and from incidental fishery takes (n = 2). Research cruises were conducted throughout the study area while all but two of the samples from strandings and fishery takes were collected in the United States and Canada. Ninety-six samples were collected from solitary individuals. One hundred ninety-one samples were collected from 50 groups in which multiple unique individuals were sampled (2–15 samples per group) (Table S2, Supporting information). In every encounter, estimates were made of group size and composition (sex and size of individuals) when possible (Table S1, Supporting information). All samples were archived in the NOAA Fisheries' SWFSC Marine Mammal and Turtle Molecular Research Sample Collection. Soft tissues were stored frozen (after 2001) in an aqueous solution of 20% dimethyl sulphoxide (DMSO) saturated with sodium chloride (NaCl) (Amos & Hoelzel 1991) or kept frozen until DNA extraction. The single tooth (collected from a stranded animal) was stored dry at room temperature.

Samples were stratified based on their geographical location (Fig. 2). Strata designations are based on the three 'population stocks' defined by the U.S. National Marine Fisheries Service (Allen & Angliss 2010; Carretta *et al.* 2010): the North Pacific (Alaska) stock, comprised predominantly of adult males and divided into areas where depredation is common (Gulf of Alaska) and uncommon (Aleutians); the California/Oregon/Washington stock (defined here as 'California Current' stratum because of the inclusion of samples from British Columbia) and the Hawai`i stock (referred to here as the 'Hawai`i stratum'). The 'eastern tropical Pacific' stratum is defined based on photographic matches by Whitehead *et al.* (2008). Samples collected outside these designated areas include samples collected near the Kuril Islands, Russia, and samples collected off the west coast of Baja California, Mexico, near Palmyra Atoll, and the open waters in between. We had no a priori basis for assigning these samples to strata so they were excluded from the population structure analysis and their affinities were examined using assignment tests.

Laboratory analyses

DNA was extracted from soft tissues using several standard methods, including lithium chloride (Gemmell & Akiyama 1996), sodium chloride protein precipitation (modified from Miller *et al.* 1988), silica-based filter purification (DNeasy kit; Qiagen, Valenica, CA, USA) and standard phenol/chloroform extraction (Sambrook *et al.* 1989). DNA extraction from the tooth was performed using a protocol developed by Rosenbaum *et al.* (1997).

Sex determination. Sex was genetically determined using the zinc finger genes (Fain & LeMay 1995), SRY genes (Richard *et al.* 1994) and after 2005, a real-time PCR assay (Morin *et al.* 2005). PCR amplifications for all protocols consisted of animals of unknown sex and two positive controls of a male and female, obtained from stranded animals for which sex was positively determined (SWFSC Marine Mammal and Turtle Molecular Research Sample Collection). Samples that failed sexing were run with alternative methods until an unambiguous result was obtained or if this was not reasonably possible, the samples were considered as sex 'unknown'.

Mitochondrial DNA. A 524-bp region of the hypervariable mitochondrial control region was amplified by PCR using primers Pmac D (5'CCTGAGAATTGCAACTAGAGG3'), which anneals to a conserved section within the control region, and Tro (5'CCTCCCTAAGACTCAAGGAAG3'), which anneals to the tRNA proline gene. Both soft tissue and tooth material were amplified in 50-μL PCR containing 1 μL (approximately 5–25 ng) genomic DNA, 1× PCR buffer [10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$], 0.3 μM of each primer, 200 μM of each dNTP and 0.5 units of *Taq* DNA polymerase. PCR cycling included an initial denaturation at 90 °C for 2 min followed by 35 cycles of 94 °C for 10 s, 48 °C for 10 s, and 72 °C for 10 s and a final extension at 72 °C for 5 min. Products were cleaned with QIAquick™ PCR purification kit (Qiagen) according to manufacturer's protocol. Sequencing of products in both directions was performed using either Big Dye™ or dRhodamine™ Terminator Cycle Sequencing kits [Applied Biosystems Inc. (ABI), Foster City, CA, USA] according to manufacturer's protocol. Products were electrophoresed on an ABI 377, 3100 or 3130XL automated sequencer. Sequences were aligned and edited using SEQED, version 1.0.3 (ABI) or SEQUENCHER software (versions 4.1 and 4.8; Genecodes, Ann Arbor, MI, USA). Throughout, approximately 10% of all samples and all rare haplotypes were re-run for quality control. If discrepancies were found within the replication, the sample was re-sequenced from extracted DNA. If the discrepancy was still not resolved, DNA was re-extracted from tissue and the sample was re-sequenced until the haplotype was confirmed.

Microsatellite genotyping, normalization of allele scores and data QC/QA. The six polymorphic dinucleotide-repeat microsatellite loci analysed in this study were amplified under similar conditions using fluorescently labelled primer sets (Table S3, Supporting information). Primers making up the D17 assay were derived from the

beluga whale (*Delphinapterus leucas*) (Buchanan *et al.* 1996), while the others were derived from sperm whale: EV1, EV5 (Valsecchi & Amos 1996) and SW10, SW13 and SW19 (Richard *et al.* 1996b). PCRs contained 1× buffer [10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$], 0.3 μM of each primer, 200 μM of each dNTP, 1.25 units *Taq* DNA polymerase and 1.0 μL of DNA in 25-μL reaction volumes. PCR cycling included an initial denaturation at 97 °C for 3 min followed by 35 cycles of 90 °C for 30 s, annealing (see below) for 1 min and a final extension at 72 °C for 1 min. The optimal annealing temperatures were 52 °C for EV1t, and 55 °C for D17t, EV5t, SW10t, SW13t and SW19t. PCR products were electrophoresed on ABI genetic analysers (models 377, 3100, 3130XL and 3730) and analysed with GENESCAN™ (version 3.1) or with ABI's GENEMAPPER (version 4.0) software. Earlier genotypes (those generated before 2004) were generated with untailed reverse primers, while later genotypes were generated with tailed primers (indicated by the 't' attached to the locus names) to reduce allelic stutter. Data from different ABI instruments and from tailed/untailed primers were normalized with the program ALLELOGRAM (Morin *et al.* 2009a). The size of each allelic pair for each locus constituted the raw data for analyses. We used control samples with each group of samples to allow us to normalize alleles to the same size bins (Morin *et al.* 2009a). Owing to the extended period of data accrual, an extensive quality control/quality analysis (QC/QA) and sample tracking procedure was implemented. In addition to standard quality control measures (e.g., running control samples, systematic sample replication), testing was carried out to ensure internal data accuracy, sample quality and to remove duplicate samples, as described by Morin *et al.* (2010) Table 2.

SNP discovery, ascertainment, genotyping and data QC/QA. Of the 36 markers analysed in this study, 16 SNPs were characterized from independent sequences and were expected to be unlinked. The other 20 SNPs consisted of eight different sets of linked loci. These linked sets consisted of two to four SNPs that were located in the same sequenced locus, often quite close together, as indicated in Table 1. The final data set consisted of 16 bi-allelic and eight multi-allelic SNP markers (after inference of linked SNP haplotypes).

Single-nucleotide polymorphisms were originally ascertained from a panel of six geographically diverse sperm whale samples and a pool of 20 geographically widespread samples (see Morin *et al.* 2007 for details). Subsequent to development of the first 18 unlinked SNP assays (two of which were nearly monomorphic in Pacific samples and not used in this study), six additional SNP assays were developed from linked SNPs in some of the same loci, so they were also ascertained from the same panel of samples. Thirty-five additional loci were then screened on 10–13 samples, resulting in ascertainment of 14 new SNP assays optimized from eight of the 35 loci (Table 1). Primers for all SNP assays are provided in Table S4 (Supporting information).

Genotyping was conducted according to the methods described by Morin & McCarthy (2007), using the Amplifluor SNP genotyping kit (Millipore, Billerica, MA, USA) and real-time detection on a MXP3000 Real-Time PCR instrument (Agilent Technologies, Santa Clara, CA, USA). Throughout, a set of seven positive control samples were replicated on each genotyping plate, and 54 samples (~18%) were replicated as genotyping controls. Replication was designed to test for both random and systematic errors (e.g., errors affecting an entire extraction plate or PCR). Testing to ensure internal data accuracy and sample quality, and to remove duplicate samples, paralleled procedures for microsatellites and are shown in Table 2.

Reconstruction of Gametic Phase for SNPs

Because some of the SNPs used in this study were discovered within close proximity to one another, they could not be treated as independent markers. For each set of linked SNP loci, we estimated the gametic phase of alleles for each sample with the Bayesian program PHASE v2.1 (Stephens *et al.* 2001). This program uses allele frequencies and frequencies of known SNP haplotypes in each population to infer the probabilities for each possible haplotype from a group of linked SNPs. For each run of PHASE, we used a burn-in of 100 iterations and a run length of 1000 iterations, with all other parameters set to their defaults. For each newly 'phased' locus, we selected the two haplotypes for each sample that had the highest probability as assessed by PHASE. These haplotypes were then used as multi-allelic genotypes for further analysis. If no genotypes had probabilities >0.5, then the genotype for that sample was considered to be unknown and treated as missing data.

In a study such as this, where the underlying population structure is not known, it is not clear whether the gametic phase for a given set of linked loci should be inferred based on all of the samples at once, or within each putative stratum. We examined the sensitivity of stratification by assessing phase based on all North Pacific samples ('North Pacific' phase), as well as within each of the three strata separately ('three strata' phase). Additionally, because PHASE infers genotypes based on their frequency in the underlying population, the algorithm may infer genotypes when data are missing in some or all of the linked loci. We examined the effect of this behaviour on our results by comparing the number of alleles and heterozygosity for phased loci where PHASE

Table 1 List of single-nucleotide polymorphism (SNP) markers and their source

Locus	SNP type	Citation	GenBank accession number	Number of linked loci and assay names
CATR 262	A/G	This study	EF087939	
CATR 456	A/G	Morin *et al.* (2007)	EF087939	(2) CATR262-R456
CHRNA1Y 111	C/T	Morin *et al.* (2007)	EF087940	(2) CHRNA1R176-Y111
CHRNA1R 76	A/G	This study	EF087940	
CKK 273	G/T	Morin *et al.* (2007)	EF087942	
CSF2R 278	A/G	Morin *et al.* (2007)	EF087946	(3) CSF2R278-K552-Y589
CSF2K 552	G/T	This study	EF087946	
CSF2Y 589	C/T	This study	EF087946	
DRD2Y 679	C/T	Morin *et al.* (2007)	EF087947	
ELN40K 209	G/T	Morin *et al.* (2007)	EF087948	
EPOR 237	A/G	This study	EF087949	
EPOY 292	C/T	Morin *et al.* (2007)	EF087949	(2) EPOR237-Y292
F9Y 80	C/T	Morin *et al.* (2007)	EF087950	
GRPY 190	C/T	Morin *et al.* (2007)	EF087956	
IFNGY 234	C/T	Morin *et al.* (2007)	EF087958	
INTS 368	C/G	Morin *et al.* (2007)	EF087960	
PKMY 237	C/T	Morin *et al.* (2007)	EF087968	
PNDR 111	A/G	Morin *et al.* (2007)	EF087969	
RDSK 456	G/T	Morin *et al.* (2007)	EF087970	
RYR2R 327	A/G	Morin *et al.* (2007)	EF087971	
SPTBN1S 279	C/G	Morin *et al.* (2007)	EF087972	(2) SPTBN1S279-Y753
SPTBN1Y 753	C/T	This study	EF087972	
PmABHD5M 274	A/C	This study	HQ609610	(3) PmABHD5M274-Y447-R671
PmABHD5Y 447	C/T	This study	HQ609610	
PmABHD5R 671	A/G	This study	HQ609610	
PmBH92S 122	C/G	This study	HQ609608	(2) PmBH92S122-Y172
PmBH92Y 172	C/T	This study	HQ609608	
PmCHYR 304	A/G	This study	EF087941 (updated)	
PmDDX5R 109	A/G	This study	HQ609609	
PmHSPA9Y 220	C/T	This study	HQ609613	
PmLAPTM4R 553	A/G	This study	HQ609611	
PmMYL4R 358	A/G	This study	EF087963 (updated)	
PmPHGDHS 172	A/C	This study	HQ609612	(4) PmPHGDHS172-M200-R223-Y321
PmPHGDHM 200	A/G	This study	HQ609612	
PmPHGDHR 223	C/G	This study	HQ609612	
PmPHGDHY 321	C/T	This study	HQ609612	

had inferred genotypes for samples with missing data ('normal' mode), and the same metrics where we considered the phased genotypes for those same samples to be missing ('keep missing data' mode). Significance was assessed by a bootstrapping approach in which we calculated the fraction of iterations in which in a randomly drawn set of the same number of samples as in our data, the number of alleles or heterozygosity in the 'normal' mode phased data was greater than, less than, or equal to the same values for the 'keep missing data' mode phased data. Tests were conducted using the 'North Pacific' and 'three strata' phase data sets.

Definition of social categories and relatedness

Each sample was also assigned a priori to one of three social categories: (i) females and young, (ii) adult males (estimated to be >13 m in size) and (iii) 'the others' comprised of males of intermediate sizes (ca. 8–12 m or the approximate size of adult females), individuals of unknown sex and males of unknown size. At sea, while it is easy to identify adult males by their large body size, it can be quite difficult to distinguish large juvenile or subadult males from adult females. Furthermore, whether males of intermediate sizes are temporary visitors or

Table 2 Sperm whale microsatellite and single-nucleotide polymorphism (SNP) quality control and quality analysis (QC/QA). Initial sample sizes between the two data sets differ because samples that were identified as duplicate or failed during microsatellite genotyping were omitted before starting SNP genotyping. The QC/QA steps below follow Table 5 in Morin *et al*. (2010) for microsatellite genotyping and data reporting

QC/QA step	Microsatellite results	SNP results
Initial sample set	325 samples typed for 8 loci	292 samples typed for 38 loci
For microsatellites: check allele binning using ALLELOGRAM (Morin *et al*. 2009a) for all experiments using control samples; for SNPs: check amplification efficiency	Could not bin alleles for loci EV37 and EV30; excluded loci from further analysis	Could not amplify ACTC380 and DRBK1; excluded loci from further analysis
Check database for genotype mismatches between replicated samples	16 of 1849 replicated genotypes (0.9%) did not match; most discrepancies were resolved by checking the raw data, others were re-genotyped	169 (58%) samples were replicated from the multiplex step at least once, resulting in a total of 4022 genotypes. 116 alleles mismatched between replicate samples. The per-allele error rate was 1.44% (116/8044 mismatched alleles/total replicate alleles). Most discrepancies were resolved by checking the raw data; others were re-genotyped
Check database for samples with ≤50% completed genotypes	28 samples had three or fewer completed genotypes and were excluded from further analysis	16 samples had <20 completed genotypes and were excluded from further analysis
Calculate percent homozygosity for all samples in database	Six samples had >50% homozygosity across six loci; five were excluded because of high failure rate and evidence of allelic dropout at several loci	Samples were ranked by percent homozygosity; the top 5% were checked by hand to look for evidence of poor quality (e.g., low-signal amplification, low number of successful genotypes). The highest percent homozygosity was 89% (one sample) and there were no clear outliers from the distribution
Test for null alleles and large allele dropout using MICROCHECKER (van Oosterhout *et al*. 2004)	Two loci have potential null alleles, but effect is limited; all loci retained for analysis	MICROCHECKER not applicable to SNP markers
Test for deviations from Hardy–Weinberg equilibrium using jackknife analysis (Morin *et al*. 2009b)	Six samples caused two markers to deviate from Hardy–Weinberg equilibrium because of homozygous rare alleles (odds ratio >2); after re-genotyping, no samples had odds ratios >2	Using 263 samples that had >20 genotyped loci, the jackknife analysis was run twice, once combining all North Pacific samples and once based on three initial strata designations. Only one sample was identified as having an anomalous genotype based on a high odds ratio (7.7), high homozygosity (86%) and seven missing genotypes. This sample was excluded from further analysis
Check for duplicate samples using DROPOUT (McKelvey & Schwartz 2004) to find multilocus genotype matches and near-matches among samples	Identified 27 perfect matches* across six loci, plus 10 potential matches; all were confirmed to be perfect matches after verifying with sex and mitochondrial DNA (mDNA). One sample of each matched set was retained and the rest were excluded from further analysis	Identified 10 sets (pairs or trios) of samples that matched† at 35–36 loci; eight of these sets were confirmed with the sex, mtDNA and microsatellite data sets to be duplicates. One sample of each matched set was retained and the rest were excluded from further analysis
Final data set	Microsatellite genotyping completed for 255 samples and six loci	SNP genotyping completed for 251 samples and 36 loci

*Microsatellites: all but one duplicate sample was taken from animals biopsied within the same group, so represent accidental replicate sampling of the same individual. At least one duplication represents potential genetic re-sampling of the same individual at different locations in different years. However, this sample was not included in those genotyped for SNPs and thus could not be confirmed as a duplicate.
†SNPs: all duplicate samples were animals biopsied from the same group, so represent accidental replicate sampling of the same individual.

Table 3 Sample sizes. For each location, the total number of separate occasions when samples were collected (from individuals or groups), the total number of unique individuals sampled, and samples sizes for each social category and for each genetic marker. See Figure 1 for sample locations and the text for strata designations. See Tables S1 and S2 (Supporting information) for additional sample information

			Number of samples collected from			Number of samples for each marker type. Total number of samples and (in parenthesis) the number of samples from females and young with potential first-order relatives omitted			
Sampling location	Number of sampling events	Number of samples	Females and young	Adult males	'Others' (subadult males and unknowns)	Mitochondrial DNA	Microsatellites	Single-nucleotide polymorphisms	Combined nuclear DNA
Temperate and tropical strata									
California Current	37	52	32	14	6	52 (31)	43 (28)	41 (28)	40 (27)
Hawai`i	19	28	24	2	2	28 (22)	27 (21)	27 (21)	26 (20)
Eastern tropical Pacific	35	115	91	15	9	114 (84)	98 (71)	102 (78)	87 (66)
Subtotal		195				194 (137)	168 (120)	170 (127)	153 (113)
North Pacific (Alaska) stock									
Aleutians	10	11	0	11	0	11	11	4	4
Gulf of Alaska	24	33	1	30	2	33	31	32	31
Other sub-Arctic									
Kuril Islands, Russia	6	6	0	6	0	6	5	6	5
Other low-latitude									
Palmyra	3	4	4	0	0	4	4	4	4
Western Baja	3	10	7	0	3	9	10	10	10
Between Hawai`i and eastern tropical Pacific	10	28	23	2	3	28	26	25	25
Total		287				285	255	251	232

members of these groups is not well known (Best 1979). We combined notes from field observations with genetic determination of sex to resolve the social status of each individual. Two data sets were compiled. 'All' contains every sample from all three social categories above and was used in subsequent analyses unless otherwise noted. 'Females and young' contains females of all size classes and young males (if they are shown to be smaller in size than adult females) and excludes one of each pair from each set of potential first-order relatives.

Because sperm whales travel in groups comprised in part of close relatives, we expected that groups from which we obtained multiple samples could contain close relatives. As long as the strata are each sampled randomly, the presence of close relatives is not necessarily a problem. However, if the putative populations differ in the number of close relatives sampled, sampling bias could lead to false detection of population structure. To avoid this potential bias, we used the maximum-likelihood program ML-RELATE (Kalinowski *et al.* 2006) to identify all potential parent–offspring pairs by identifying samples that shared at least one allele at every microsatellite and at every SNP locus. We excluded one of each pair from the 'females and young' data set.

Molecular diversity

For the mtDNA, haplotypic diversity (h) (the probability that two randomly chosen haplotypes are different) and nucleotide diversity (π) (the probability that two randomly chosen homologous sites are different) (Nei 1987) were calculated using ARLEQUIN 3.11 (Excoffier *et al.* 2005). For each microsatellite and SNP locus, allele frequencies were calculated using GENEPOP version 4.0 (Rousset 2008) and allelic richness was calculated using FSTAT (Goudet 1995). For each stratum, the mean number of alleles and observed heterozygosity and expected heterozygosity were calculated using ARLEQUIN 3.11 (Excoffier *et al.* 2005).

Deviations from Hardy–Weinberg equilibrium (HWE) were assessed for each locus in each stratum using GENEPOP version 4.0 (Rousset 2008). Both the probability test of Guo & Thompson (1992) and tests for heterozygote deficiency (Rousset & Raymond 1995) were conducted using program defaults for the Markov chain parameters (1000 dememorization steps, 100 batches, 5000 iterations per batch). Tests were conducted for all samples combined and then separately for each of the three strata. Global significance was assessed using Fisher's method and a sequential Bonferroni correction (Simes 1986; Hochberg 1988) was applied across all tests conducted for each stratum. For the SNP data, tests are reported for the data set used in the population structure analysis described below (24 loci with haplotypic inference based on 'three strata' phase data set; AS82b). GENEPOP version 4.0 was also used to evaluate linkage disequilibrium (LD) (Weir 1996) between each pair of loci. As above, tests were conducted for all samples combined and then separately for each of the three strata, using Fisher's method. For the SNP data, we used the 24 loci used in the population structure analyses and we also report results for LD tests evaluated using the 36 bi-allelic SNP alleles.

Population differentiation

We first tested the global null hypothesis of panmixia among the three low-latitude strata containing females (California Current, Hawai`i and eastern tropical Pacific) for the mtDNA, microsatellite and SNP data sets separately, and for a combined nuclear (nDNA) data set comprised of both the microsatellite and SNP data sets. Comparisons were then conducted between all pairs of strata. Differentiation for the mtDNA data set was assessed using F_{ST} and Φ_S (Weir & Cockerham 1984; Excoffier *et al.* 1992) and Fisher's exact test (Raymond & Rousset 1995; Goudet *et al.* 1996) as implemented in the program ARLEQUIN version 3.11 (Excoffier *et al.* 2005). Φ_{ST} was calculated using the nucleotide substitution model receiving the highest AIC score in jMODELTEST (Guindon & Gascuel 2003; Posada 2008). Statistical significance was determined using 16 000 random permutations of the original data sets for F_{ST} and Φ_{ST} and using 10 000 replications for Fisher's exact test. Below, we present the results for F_{ST} and for Fisher's exact test, which has been found to be more powerful than F_{ST} and Φ_{ST} permutation tests for evaluating statistical significance in mtDNA (Hudson *et al.* 1992). The results for all tests performed on the mtDNA data are shown in Table S5 (Supporting information).

We examined the extent of genetic differentiation for the microsatellite, SNP and combined nDNA data sets using custom-written code in the statistical programming language R (R Development Core Team 2005) (available upon request, EIA). The following tests were performed: F_{ST} (Weir & Cockerham 1984), F'_{ST} (Hedrick 2005; Meirmans & Hedrick 2010), G_{ST} (Nei 1973), G'_{ST} (Hedrick 2005), G''_{ST} (Meirmans & Hedrick 2010), D (Chao *et al.* 2008) (as presented in Jost (2009) Equation 13) and χ^2 (Roff & Bentzen 1989). Statistical significance was determined through 10 000 random permutations of the original data set. Although F_{ST} and G_{ST} are widely used as measures of population differentiation for nuclear data sets, they have been criticized recently because of their dependency on within-population diversity (Hedrick 2005; Jost 2008, 2009; Heller & Siegismund 2009; Ryman & Leimar 2009; Meirmans & Hedrick 2010). To correct for this bias, several replacement statistics have been proposed, and it is recommended that they be used in combination with traditional measures because all have their advantages and drawbacks (Meirmans & Hedrick 2010). The results for all tests performed on the nuclear data sets are reported in Table S5 (Supporting information). In the tables below, we present the results for F_{ST} and F'_{ST}. F'_{ST} (calculated by standardizing the normal F_{ST} by the maximum value it can obtain given the observed within-population diversity) is considered to provide a more accurate estimate of the degree of divergence between populations (Meirmans & Hedrick 2010).

We assessed genetic differentiation between the males in the North Pacific (Alaska) stock with the three low-latitude strata that contain females for the nuclear markers that resulted in the highest sample size (and hence statistical power). All pairwise comparisons, therefore, of Alaska males to the low-latitude strata were examined using SNPs (the markers that yielded the highest sample size) and using all samples collected in the low-latitude strata. Two comparisons were made: Gulf of Alaska males (where depredation is known to be frequent) and all Alaska males together.

Assignment

Individual assignment likelihoods and the probability of exclusion to each of the three putative source populations were investigated using GENECLASS2 (version 2.0) (Piry *et al.* 2004). We used the combined nDNA data set comprised of microsatellites and SNPs (to maximize the number of loci used to estimate assignment) and the assignment criterion of Paetkau *et al.* (1995) to calculate the assignment likelihoods and assess the significance of those likelihoods using Paetkau *et al.*'s (2004) re-sampling method. Reference samples from the three potential source populations (California Current, Hawai`i and eastern tropical Pacific) were comprised only of samples from 'females and young' (n = 113) to avoid inclusion of potentially nonresident males. We calculated probabilities of origin from each of the source populations for (i)

California Current, Hawai`i and eastern tropical Pacific females and young using themselves as the potential source populations ('self-assignment'/detection of migrants), (ii) assignment of adult males sampled from within these three strata and from sub-Arctic waters to the three potential source populations comprised of females and young and (iii) assignment of groups comprised predominantly of females and young sampled in waters outside the three strata (samples collected in Palmyra, western Baja California and the open waters in between; Fig. 2). For all analyses, we set the default frequency for missing alleles at 0.01, performed 1000 resampling events and set the type-1 error rate to 0.01 as recommended by Piry *et al.* (2004).

Results

The final genetic data sets, quality checked for common genotyping problems following recommendations described by Morin *et al.* (2010) and with duplicate and poorest-quality samples removed, consisted of mtDNA (n = 285), six microsatellites (n = 255), 36 SNPs (n = 251), and combined nDNA (six microsatellites and 36 SNPs) (n = 232). The per-allele genotyping error rate for microsatellites was 0.9% and 1.4% for SNPs. Sex was determined genetically and/or by field examination of stranded animals for 272 samples. Genotypic data are available online (Dryad entry DOI: 10.5061/dryad.7983). Sample sizes for each strata, each of the marker types and for the social categories are shown in Table 3.

Reconstruction of gametic phase for SNPs

Reconstruction of the gametic phase for linked SNP loci resulted in a data set comprised of 16 bi-allelic loci and eight multi-allelic loci (with two to four linked SNPs each), for a total of 24 SNP loci. The difference between assessing phase based on all North Pacific samples and that of assessing phase based on the 'three strata' separately on the results of the population structure analyses was nearly indistinguishable (Table S5, Supporting information; compare AS82a and AS82b). The comparison of 'normal' and 'keep missing data' modes of PHASE resulted in relatively few samples with more missing data. In the data set comprised of samples from the three strata (n = 170 samples), for example, the number of missing data increased from 222 alleles in the 'normal' mode data set to 308 in the 'keep missing data' mode. The bootstrap analysis revealed no significant deviations from random for the number of alleles calculated at each locus for either the full or three strata data sets. The bootstrap analysis showed significant differences in heterozygosity between the two modes but in opposite directions (one locus in one stratum showed higher heterozygosity in 'normal' mode while two loci in a second stratum showed higher heterozygosity in the 'keep missing data' mode; data not shown). The difference between the results of population structure analyses estimated in the two modes was nearly indistinguishable as shown in Table S5 (Supporting information) [compare 'three strata' results (AS82b) with those of 'three strata', 'keep missing data' (AS82e)]. The difference in increasing the number of PHASE iterations from 1000 to 1 000 000 had little influence on the results of the population structure statistics when comparing the SNP data set (AS82b) with 'three strata' phase and 'normal' mode (data not shown). Because of the small number of differences described above among phase data sets, all molecular diversity and population structure results shown below will be presented using 'normal' mode, 'three strata' stratification of the data set and 10 000 iterations of PHASE for the SNP data sets and combined nDNA data sets (AS82b and AS84b, respectively, in Table S5, Supporting information) (unless stated otherwise).

Molecular diversity

For the mtDNA sequences, 24 sites were variable and 17 different haplotypes identified (Table S6, Supporting information), seven of which were unique to one of the three low-latitude strata (Table 4). All haplotypes were defined by transitions. Among the three strata, nucleotide diversity was low (0.2–0.4%) and haplotypic diversity ranged from 0.643 to 0.833 (Table 6). Haplotypic diversity was lowest in the samples from Hawai`i (Table 5).

The number of microsatellite alleles per locus ranged from nine for locus EV5 to 26 for SW19 (Table S7, Supporting information). Summaries of allelic diversity by strata and by locus are presented in Table 6a,b, respectively. For the three strata, heterozygosity ranged from 0.800 in the California Current to 0.832 in Hawai`i. When all samples were combined into a single population, the probability test detected no deviations from HWE for the global comparison and no deviations from HWE were detected for any loci, or for any loci in the strata-specific tests. The test for heterozygote deficiency was significant for the global comparison but no single locus showed significant deviations from HWE. Once the samples were divided into strata, SW10 exhibited heterozygote deficiency in the California Current stratum; no significant deviations from HWE were detected for any other loci in any of the other strata. No pairs of loci showed statistically significant linkage disequilibrium.

The number of SNP alleles ranged from two for the bi-allelic loci to nine for the linked sets of loci (Table S8, Supporting information). Summaries of allelic diversity by strata and by locus are presented in Table 6c,d, respec-

tively. All SNP loci were polymorphic in each of the three strata, except for locus GRPY190 that was monomorphic in Hawai`i (Table 6d, Table S8, Supporting information). For the three strata, heterozygosity ranged from 0.391 in the eastern tropical Pacific to 0.426 in the California Current (Table 6c). Among loci, heterozygosity varied, with GRPY190 and PmCHYR304 being the least heterozygous (Table 6d and Table S8, Supporting information). The same two loci showed minor allele frequencies (MAF) <0.05 as did two of the linked loci CATR262_456 and CSF2KR278-K552-Y589; otherwise, minor allele frequencies were >0.05 (Table S8, Supporting information). When all three strata were combined into a single population, the probability test detected no deviation from HWE for the global comparison and no deviations for any loci, or for any loci in the strata-specific tests. The test for heterozygote deficiency was significant for the global comparison but no single locus showed significant deviations from HWE. Once the samples were divided into the three strata, no deviations from HWE were detected for any loci in any of the strata. Tests of linkage disequilibrium were investigated on two different data sets. In the first test, we examined linkage between all possible pairs of the 36 original bi-allelic markers over all samples (n = 251). Of the possible 630 locus combinations, 14 pairs showed significant disequilibrium; eight of these pairs were between known linked loci. In the second test, we examined linkage between all possible combinations for the 24 loci in the three strata shown in Table 6d (n = 170). Of the possible 276 pairwise comparisons between loci, 11 showed statistically significant linkage disequilibrium ($P < 0.05$). In a third test, we used the same data set but examined linkage between all possible pairs of loci within each of the three strata. Of the possible 276 pairwise comparisons in each of the three strata, 11, 11 and 15 pairs of loci showed statistically significant linkage disequilibrium ($P < 0.05$) in California Current, Hawai`i and eastern tropical Pacific strata, respectively. In only two cases were pairs of loci that showed significant LD in one stratum, also significant in another stratum; none showed significant linkage disequilibrium in all three strata. As there was no consistent pattern, we chose to treat loci for which we had no prior indication of physical linkage as independent.

Table 4 Number of haplotypes found in each stratum

Haplotype	California Current (n = 52)	Hawai`i (n = 28)	Eastern tropical Pacific (n = 114)
a	15	15	38
aa	2	0	0
b	10	6	39
c	6	0	10
d	0	0	3
e	11	1	2
f	1	0	4
g	1	0	0
h	2	0	3
i	1	0	0
j	0	6	11
k	0	0	0
l	0	0	1
m	1	0	0
n	1	0	1
o	0	0	1
p	1	0	1

Table 5 Mitochondrial DNA haplotypic and nucleotide diversity (± standard deviation) for the three strata

Strata	Sample size	Number of haplotypes	Haplotypic diversity	Nucleotide diversity
California Current	52	12	0.833 ± 0.028	0.004 ± 0.003
Hawai`i	28	4	0.643 ± 0.068	0.002 ± 0.002
Eastern tropical Pacific	114	12	0.758 ± 0.025	0.003 ± 0.002

Population structure

Global tests revealed significant genetic differentiation among strata for mtDNA and all nDNA data sets, rejecting the global null hypothesis of no population structure for the three strata (Table S5, Supporting information). Results were significant ($P < 0.05$) for mtDNA (F_{ST}, Φ_{ST} and Fisher's exact test); microsatellites (χ^2); and all SNP and combined nDNA data sets (F_{ST}, F'_{ST}, G'_{ST}, G'_{ST}, G''_{ST}, D and χ^2) (Table S5, Supporting information). For the mtDNA data, analysis of molecular variance (AMOVA) revealed that most of the genetic variance was within populations (96.14% for F_{ST}; 96.24% for Φ_{ST}). The remaining molecular variance was found among populations (3.86% for F_{ST}; 3.76% for Φ_{ST}).

Demographic independence of the three strata

Pairwise tests using mtDNA revealed significant genetic differentiation ($P < 0.05$) among all three strata (F_{ST}) and for pairwise comparisons between California Current and Hawai`i and between California Current and eastern tropical Pacific (Fisher's exact test) (Table 7). The only significant pairwise difference for the microsatellite data set was between California Current and eastern tropical Pacific for χ^2 (P = 0.03; Table S5, Supporting information). All pairwise com-

Table 6 Estimates of the number of alleles, observed (H_o) and expected (H_e) heterozygosity, and allelic richness for the nuclear loci: microsatellites averaged over strata (a) and for each locus (b); single-nucleotide polymorphisms (SNPs) averaged over strata (c) and for each locus (d)

(a) Microsatellite diversity by strata

Strata	Sample size	Mean number of alleles	Mean H_o	Mean H_e
California Current	43	11.8 ± 4.6	0.800 ± 0.115	0.813 ± 0.105
Hawai`i	27	11.0 ± 3.6	0.832 ± 0.090	0.832 ± 0.114
Eastern tropical Pacific	98	14.7 ± 5.1	0.814 ± 0.076	0.819 ± 0.102

(b) Microsatellite diversity by locus

	California Current				Hawai`i				Eastern tropical Pacific			
	Number of alleles	H_o	H_e	Allelic richness	Number of alleles	H_o	H_e	Allelic richness	Number of alleles	H_o	H_e	Allelic richness
D17	17	0.952	0.889	14.725	13	0.889	0.901	12.887	20	0.898	0.921	15.456
EV1	7	0.643	0.643	6.908	7	0.667	0.621	6.962	12	0.755	0.669	7.949
EV5	8	0.721	0.726	7.217	8	0.815	0.780	7.888	9	0.773	0.737	7.335
SW10	11	0.767	0.849	9.984	11	0.852	0.898	10.925	13	0.827	0.862	10.098
SW13	10	0.816	0.855	9.042	10	0.846	0.869	10.000	12	0.725	0.804	9.105
SW19	18	0.902	0.913	15.821	17	0.926	0.920	16.811	22	0.904	0.919	14.936

(c) SNP diversity by strata

Strata	Sample size	Mean number of alleles	Mean H_o	Mean H_e
California Current	41	2.6 ± 1.1	0.427 ± 0.204	0.423 ± 0.182
Hawai`i	27	2.5 ± 1.0	0.420 ± 0.205	0.414 ± 0.197
Eastern tropical Pacific	102	2.7 ± 1.3	0.392 ± 0.191	0.409 ± 0.191

(d) SNP diversity by locus

	California Current				Hawai`i				Eastern tropical Pacific			
	Number of alleles	H_o	H_e	Allelic richness	Number of alleles	H_o	H_e	Allelic richness	Number of alleles	H_o	H_e	Allelic richness
CATR262-R456	3	0.268	0.363	2.97	4	0.462	0.439	3.85	4	0.382	0.387	3.60
CHRNA1R76-Y111	3	0.488	0.485	3.00	3	0.630	0.475	2.99	3	0.294	0.343	2.92
CSF2R278-K552-Y589	4	0.537	0.593	3.83	4	0.704	0.680	3.89	4	0.743	0.672	3.94
EPOR237-Y292	3	0.610	0.548	3.00	3	0.370	0.414	3.00	3	0.382	0.426	3.00
PmABHD5M274-Y447-R671	4	0.756	0.677	3.83	4	0.593	0.663	3.89	4	0.590	0.671	3.92
PmBH92Y172	3	0.756	0.661	3.00	3	0.519	0.610	3.01	3	0.673	0.660	3.00
PmPHGDHS172-M200-R223-Y321	7	0.781	0.795	6.17	5	0.741	0.744	5.01	8	0.686	0.775	5.70
SPTBN1S279-Y753	3	0.415	0.402	3.00	3	0.185	0.175	3.00	3	0.373	0.334	2.98
CKK273	2	0.500	0.461	2.00	2	0.440	0.507	2.00	2	0.467	0.503	2.00
DRD2Y679	2	0.512	0.505	2.00	2	0.519	0.509	2.00	2	0.539	0.502	2.00
ELN40K209	2	0.463	0.409	2.00	2	0.520	0.507	2.00	2	0.480	0.499	2.00
F9Y80	2	0.325	0.481	2.00	2	0.440	0.481	2.00	2	0.458	0.501	2.00
GRPY190	2	0.098	0.094	1.97	0	0.000	0.000	1.00	2	0.071	0.069	1.86
IFNGY234	2	0.205	0.187	2.00	2	0.154	0.149	2.00	2	0.110	0.156	1.99
INTS368	2	0.375	0.481	2.00	2	0.630	0.507	2.00	2	0.542	0.473	2.00
PKMY237	2	0.342	0.287	2.00	2	0.185	0.171	2.00	2	0.287	0.325	2.00

Table 6 *Continued*

(d) SNP diversity by locus

	California Current				Hawai`i				Eastern tropical Pacific			
	Number of alleles	H_o	H_e	Allelic richness	Number of alleles	H_o	H_e	Allelic richness	Number of alleles	H_o	H_e	Allelic richness
PmCHYR304	2	0.075	0.073	1.94	2	0.074	0.073	2.00	2	0.062	0.060	1.82
PmDDX5R109	2	0.316	0.438	2.00	2	0.333	0.479	2.00	2	0.441	0.469	2.00
PmHSPA9Y220	2	0.220	0.235	2.00	2	0.111	0.107	2.00	2	0.090	0.122	1.98
PmLAPTM4R553	2	0.125	0.162	2.00	2	0.259	0.283	2.00	2	0.247	0.248	2.00
PmMYL4R358	2	0.512	0.476	2.00	2	0.519	0.475	2.00	2	0.366	0.446	2.00
PNDR111	2	0.684	0.507	2.00	2	0.615	0.507	2.00	2	0.441	0.500	2.00
RDS456	2	0.375	0.339	2.00	2	0.111	0.107	2.00	2	0.270	0.263	2.00
RYR2R327	2	0.500	0.504	2.00	2	0.539	0.462	2.00	2	0.409	0.423	2.00

parisons with the California Current stratum were significant for both the SNP and combined nDNA data sets ($P \leq 0.01$; F_{ST} and F'_{ST}; Table 7). No comparisons between the Hawai`i and the eastern tropical Pacific were significant for either of these marker sets. These results are consistent with the results from the additional data sets and statistical tests examined in Table S5 (Supporting information). Pairwise mtDNA F_{ST} values ranged from 0.033 to 0.070 (Table 7). Microsatellite F_{ST} values ranged from 0.001 to 0.003 and SNP F_{ST} values ranged from 0.001 to 0.013 (Table 7). Analysis of genetic differentiation among the three strata using the restricted 'females and young' data set were similar to those described above, although some comparisons became marginal or nonsignificant (compare results in Table 7 to Table S9, Supporting information).

Assignment of low-latitude samples

Despite the significant differentiation among strata, on a per individual basis, there is little power for assignment or exclusion to the three source strata. For females and young, the proportion of 'self-assignments' (samples collected in one of the three designated source strata that assigned most strongly to their stratum of origin) was 44% (12/27) for California Current, 50% (33/66) for the eastern tropical Pacific and 30% (6/20) for Hawai`i (Fig. 3). Individuals in the Hawai`i stratum tended to show higher assignment likelihoods to the eastern tropical Pacific stratum than to Hawai`i. One individual from the eastern tropical Pacific and one individual from Hawai`i could be excluded from their source stratum (exclusion probability <0.01) (shown in solid black in Fig. 3).

For females and young from areas outside the three designated strata (samples collected in Palmyra, western Baja California and the open waters in between; Fig. 2), few individuals could be excluded from any of the three putative source strata. The source strata to which they had the highest assignment likelihood were mixed (data not shown).

Adult males from the three strata showed mixed assignments and relatively few exclusions (Table 8). The proportion of samples that assigned most strongly to their stratum of origin was 66% (4/6) for California Current, 56% (5/9) for eastern tropical Pacific and 0% (0/2) for Hawai`i.

Single or mixed population status of Alaska and males

All pairwise comparisons of males from the North Pacific (Alaska) stock to the low-latitude strata using SNPs (the markers that yielded the highest sample size) were statistically significant or nearly so, suggesting that these males do not originate solely from only one of the three populations (F'_{ST} *P*-values for comparisons between Alaska and California Current = 0.07; Alaska and eastern tropical Pacific = 0.02; and Alaska and Hawai`i = 0.05). Results were similar with mtDNA ($F_{ST}$ *P*-values for comparisons between Alaska and California Current = 0.04; Alaska and eastern tropical Pacific = 0.01; and Alaska and Hawai`i = 0.17). Results were nearly identical for both markers in the comparison including only samples of males from the Gulf of Alaska (where depredation is common) to the three low-latitude strata (Table S10, Supporting information). For all but two of the Gulf of Alaska males and for all but one of the four Aleutian males, none of the three putative source strata could be excluded as a possible source population (exclusion probability ≥0.01; Table 8). Samples of males collected along the Kuril Islands, Russia (n = 5), showed mixed assignments, and one individual could be excluded from all three putative source populations (Table 8).

Table 7 Pairwise divergence between strata. Comparisons that are statistically significant at the $\alpha = 0.05$ level are in bold. For mitochondrial DNA (mtDNA) sequence data (a) divergence was estimated with F_{ST} (with *P*-values in parenthesis) below the diagonal and for Fisher's exact test *P*-values above the diagonal. $N = 194$ (AS 108). For the nuclear data, divergence was estimated with F'_{ST} (above the diagonal) and F_{ST} (below the diagonal) with *P*-values (in parenthesis below the diagonal). For microsatellite data (b) $n = 168$ (AS78). For SNP data (c) $n = 170$ (AS82b). For the combined nuclear DNA (nDNA) (d) $n = 153$ (AS84b)

(a) mtDNA	California Current ($n = 52$)	Hawai`i ($n = 28$)	Eastern tropical Pacific ($n = 114$)
California Current	—	**$P = 0.001 \pm 0.001$**	**$P < 0.001$**
Hawai`i	**0.070 ($P = 0.005 \pm 0.001$)**	—	$P = 0.291 \pm 0.015$
Eastern tropical Pacific	**0.034 ($P = 0.008 \pm 0.001$)**	**0.033 ($P = 0.047 \pm 0.002$)**	—

(b) Microsatellites	California Current ($n = 43$)	Hawai`i ($n = 27$)	Eastern tropical Pacific ($n = 98$)
California Current	—	0.017	0.013
Hawai`i	0.003 ($P = 0.194$)	—	0.006
Eastern tropical Pacific	0.002 ($P = 0.119$)	0.001 ($P = 0.318$)	—

(c) SNPs	California Current ($n = 41$)	Hawai`i ($n = 27$)	Eastern tropical Pacific ($n = 102$)
California Current	—	**0.018**	**0.022**
Hawai`i	**0.010 ($P = 0.016$)**	—	0.001
Eastern tropical Pacific	**0.013 ($P < 0.001$)**	0.001 ($P = 0.378$)	—

(d) Combined nDNA	California Current ($n = 40$)	Hawai`i ($n = 26$)	Eastern tropical Pacific ($n = 87$)
California Current	—	**0.018**	**0.018**
Hawai`i	**0.009 ($P = 0.007$)**	—	0.003
Eastern tropical Pacific	**0.009 ($P < 0.001$)**	0.001 ($P = 0.289$)	—

Discussion

Both mtDNA and nDNA indicate population structure within the eastern and central North Pacific (questions 1 and 2 are yes). The California Current stratum showed low but significant differentiation from animals sampled both in adjacent waters to the south and in distant waters to the west in Hawai`i. These results are the first to reveal genetic differentiation in the eastern Pacific Ocean, where no physical barriers exist among geographically defined strata, and contrast with previous results in the Atlantic Ocean (Bond 1999; Engelhaupt *et al.* 2009) and the Pacific Ocean (Dillon 1996; Lyrholm & Gyllensten 1998; Lyrholm *et al.* 1999). The results are robust to uncertainties about social structure. Analysis of genetic differentiation among the three strata using the restricted 'females and young' data set was similar to that described above. Higher *P*-values using the restricted data set are not surprising given the reduced sample sizes but are contrary to what might be expected if adult males had no fidelity to their natal strata. F_{ST} values inferred from mtDNA sequences were higher than estimates for nuclear-derived microsatellites or SNPs, as is expected owing to the fourfold higher effective population size of nuclear markers compared to mitochondrial loci. However, significant differentiation in the SNPs is the first positive indication that more males (than random expectation) return to their stratum of origin to mate. Estimates of F_{ST} were greater than expected for gene-flow rates of one migrant per generation (Box 1) for all pairwise comparisons for mtDNA and the combined nDNA even given the uncertainty in N_e. The low levels of genetic differentiation in mtDNA revealed here are consistent with those found in previous studies of mtDNA nucleotide variation at regional and global scales. Our microsatellite results are also consistent with previous studies that tend to indicate either no or marginal significance for within-ocean basin comparisons using a larger number of loci (Bond 1999; Lyrholm *et al.* 1999; Engelhaupt *et al.* 2009).

Pairwise comparisons of the Hawai`i stratum to the eastern tropical Pacific stratum are not conclusive at this stage. Comparisons with the mtDNA data set are significant, but nDNA data sets cannot reject the hypothesis that there is one large stock in the eastern tropical Pacific

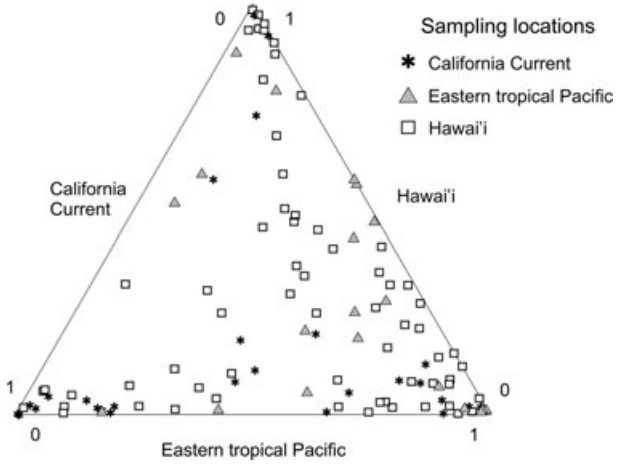

Fig. 3 Probability of 'self-assignment'/detection of migrants to three source strata for females and young from California Current, eastern tropical Pacific and Hawai`i strata (n = 113). Samples that can be excluded from their strata of origin (exclusion probabilities ≤0.01) are shown as a solid black triangle and solid black square (located in the lower left of the graph).

spanning these waters (question 1 is yes for mtDNA; cannot reject the null hypothesis for nDNA for question 2). However, there is likely to be low statistical power to detect differentiation owing to the small sample size in Hawai`i. And while haplotypic diversity was lower in Hawai`i than the other two strata, heterozygosity was not, for either microsatellites or SNPs. The discrepancy in the two markers may be attributed to the lower effective population size of mtDNA markers, compared to nuclear markers, which results in greater estimates of divergence for mtDNA (Larsson *et al.* 2009; Martien *et al.* 2010). There were few high probabilities of self-assignment, particularly of Hawaiian females, but this is not unexpected given the low individual power of assignment, the possibility of mixed parentage and un-sampled source populations, and the potential for long-range movement. Increasing the number of samples and the number of markers will be important in helping to resolve questions of population differentiation and assignment within and between Hawai`i and the eastern tropical Pacific. Outside the eastern tropical Pacific (Whitehead *et al.* 2008), we know little of the ranging patters of females. Whalers reported calving in Hawai`i year-round, and acoustic data from Hawaiian waters show a year-round low-level presence of sperm whales (Thompson & Friedl 1982). Another factor that may obscure geographical patterns of genetic differentiation between the two geographical regions is the social structure of female sperm whales. Groups in the eastern tropical Pacific and South Pacific are known to be structured into sympatric acoustic clans, and investigations using mtDNA suggest that the clans may be genetically differentiated (Rendell & Whitehead 2003; L. Rendell, S. L. Mesnick, M. Dalebout, J. Burtenshaw & H. Whitehead, in submitted). Ultimately, data from genetics, acoustics, photo-identification and tagging will need to be combined to provide a better understanding of the scale and the factors contributing to the structure of female groups.

If all or most of the Alaska males originated from only one of the three low latitude strata, then we would expect to obtain nonsignificant results when comparing these males with that stratum. Based on both mtDNA and SNPs, however, we found no strong evidence indicating that Alaska males originate solely from any one of the three strata (F_{ST} and F'_{ST} P-values significant or nearly so for comparisons between the Alaska males and each of the three strata; question 3 is no; with similar results for the Gulf of Alaska males assessed separately). Taken together with the population structure results described above, it appears that some males return to the region of their birth to mate but share high latitude feeding grounds with males from other populations.

With the low power of individual assignment tests, few Gulf of Alaska or Aleutian males could be excluded from any of the three putative source strata (question 4), limiting the utility of the assignment tests in evaluating the origin of these individuals.

As expected (Box 1), the amount of genetic differentiation among strata is low. The results found in the present study, however, using both mtDNA and nDNA, are indicative of demographic independence of the California Current stratum and provide some support for the demographic independence of the Hawai`i stock with mtDNA. Taken together with the results described above for adult males, it appears that the North Pacific (Alaska) stock is likely to have widespread origin and is probably a mix of males from multiple demographically independent populations of females. Currently, the Alaska stock is the only stock managed under the MMPA known to be comprised almost entirely of a single demographic component, i.e., adult males. Our results suggest that Alaska males appear to constitute a mixed stock on sub-Arctic foraging grounds, and their status warrants reconsideration under the MMPA. This will require further research and analysis as sample sizes are low and not all areas with females have been sampled.

Although the genetic power for individual SNPs is lower than that of individual microsatellites because they are bi-allelic, we found here that the set of SNPs examined here can provide sufficient power to detect population structure even when divergence between populations is very low. There is still work to be carried out to develop better ways to analyse SNP data, especially when linked SNPs are used. At least for this data set, however, we have shown that current methods for haplotype inference do not appear to be significantly

Table 8 Maximum assignment likelihoods and exclusion probabilities for adult males to three putative source populations (California Current, Eastern tropical Pacific and Hawai`i). Exclusion probabilities ≤0.01 are indicated in bold

Sampling location	LABID	Strongest assignment	Exclusion probability: CA Current	Exclusion probability: Hawai`i	Exclusion probability: eastern tropical Pacific
Adult males sampled within the three strata					
CA-OR-WA	2181	HI	0.759	0.973	0.963
CA-OR-WA	5753	HI	0.626	0.606	0.534
CA-OR-WA	5754	CA-OR-WA	0.056	0.016	**0.012**
CA-OR-WA	6223	CA-OR-WA	0.183	0.078	0.032
CA-OR-WA	17131	CA-OR-WA	0.767	0.375	0.262
CA-OR-WA	25442	CA-OR-WA	0.949	0.956	0.917
ETP	9598	ETP	0.218	0.365	0.251
ETP	11574	ETP	0.124	0.316	0.052
ETP	15042	HI	0.092	0.473	0.587
ETP	15966	ETP	0.204	0.380	0.218
ETP	60597	ETP	0.151	0.635	0.261
ETP	60599	CA-OR-WA	0.176	0.131	0.017
ETP	60601	CA-OR-WA	0.142	0.096	0.031
ETP	60602	ETP	0.017	0.047	0.022
ETP	60604	CA-OR-WA	0.669	0.492	0.330
HI	30488	CA-OR-WA	0.555	0.474	0.459
HI	30482	CA-OR-WA	0.033	**0.006**	0.023
Adult males sampled in sub-Arctic waters					
Gulf of AK	4848	CA-OR-WA	0.886	0.807	0.177
Gulf of AK	10399	CA-OR-WA	0.212	0.094	0.013
Gulf of AK	10400	CA-OR-WA	0.173	0.102	0.078
Gulf of AK	28393	HI	0.76	0.816	0.701
Gulf of AK	28399	CA-OR-WA	0.036	0.017	**0.006**
Gulf of AK	28411	CA-OR-WA	0.474	0.406	0.363
Gulf of AK	34557	CA-OR-WA	0.54	0.226	0.027
Gulf of AK	34558	HI	0.153	0.438	0.348
Gulf of AK	34559	HI	0.164	0.201	0.315
Gulf of AK	34562	ETP	0.245	0.450	0.266
Gulf of AK	35340	ETP	0.020	0.014	0.022
Gulf of AK	41462	CA-OR-WA	0.334	0.359	0.263
Gulf of AK	41492	CA-OR-WA	0.531	0.514	0.315
Gulf of AK	43541	ETP	0.039	0.202	0.110
Gulf of AK	43542	ETP	0.926	0.985	0.946
Gulf of AK	43543	CA-OR-WA	0.668	0.601	0.403
Gulf of AK	43544	CA-OR-WA	0.531	0.394	0.146
Gulf of AK	43749	ETP	0.149	0.452	0.291
Gulf of AK	43750	ETP	0.134	0.299	0.118
Gulf of AK	44015	HI	0.561	0.721	0.593
Gulf of AK	44016	HI	0.226	0.331	0.362
Gulf of AK	45756	HI	0.099	0.145	0.221
Gulf of AK	45757	CA-OR-WA	0.429	0.452	0.162
Gulf of AK	45758	CA-OR-WA	0.887	0.854	0.233
Gulf of AK	52892	CA-OR-WA	0.476	0.384	0.190
Gulf of AK	60878	ETP	0.338	0.570	0.179
Gulf of AK	60879	HI	0.700	0.892	0.875
Gulf of AK	60880	ETP	0.021	0.309	0.074
Gulf of AK	67880	CA-OR-WA	0.901	0.902	0.743
Gulf of AK	68973	CA-OR-WA	0.068	0.044	**0.004**
Aleutians	28556	CA-OR-WA	0.595	0.598	0.302
Aleutians	28557	CA-OR-WA	0.020	**0.012**	**0.005**
Aleutians	28559	ETP	0.052	0.083	0.036

Table 8 *Continued*

Sampling location	LABID	Strongest assignment	Exclusion probability: CA Current	Exclusion probability: Hawai`i	Exclusion probability: eastern tropical Pacific
Aleutians	57917	HI	0.087	0.180	0.192
Russia	70555	HI	0.276	0.371	0.294
Russia	70556	CA-OR-WA	0.504	0.169	0.130
Russia	70558	CA-OR-WA	0.409	0.351	0.253
Russia	70559	ETP	**0.000**	**0.001**	**0.001**
Russia	70560	ETP	0.186	0.453	0.210

biased. We used the program PHASE to infer the gametic phase for sets of linked SNPs, and we evaluated the effect of phasing SNPs based on different populations and of using haplotypes inferred from missing data. We found that, although both introduce some noise and potential bias, in the case of this data set, the inference of population structure for eastern and central North Pacific sperm whales is not affected.

While we detected significant divergence between strata, on a per individual basis, there was little power for assignment, even with the combined nDNA data set. This is not unexpected owing to the very low levels of differentiation between strata and the fact that we have not sampled all potential source populations from which migrants may have originated. While assignment power can be improved by using a larger sample (a sample size near 50 individuals is suggested for large populations) and by doubling the number of (multi-allelic) loci (Paetkau *et al.* 2004), individual assignment will remain challenging given the relatively low divergence among these strata.

Single-nucleotide polymorphisms are rapidly becoming the marker of choice for some types of population genetic studies. While SNPs discovery and ascertainment take significant time at present, and other markers may currently be better suited for particular questions such as assignment or relatedness, there are advantages that make SNPs a good marker choice. These include a lower and simple mutation model, high abundance in most genomes, highly efficient and accurate genotyping methods, sufficient statistical power with a two- to fourfold increase in markers and/or use of linked loci, and the ability to easily generate and combine data from different technologies and over long time periods (Kuhner *et al.* 2000; Turakulov & Easteal 2003; Aitken *et al.* 2004; Morin *et al.* 2004, 2009c; Seddon *et al.* 2005; Anderson & Garza 2006; Morin & McCarthy 2007; Smith & Seeb 2008). This study is one of the first to utilize SNP markers to study a widely dispersed, highly mobile marine species where we have little a priori indication of population structure. The challenges that we face in understanding population structure in sperm whales are significant, including the collection of samples over many decades and the future need to combine data from researchers with access to samples in different oceanic regions. SNPs appear to offer the best option so far for overcoming those challenges and serve as a foundation for future regional and global collaborations.

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Conflict of Interest

The authors have no conflict of interest to declare and note that the sponsors of the issue had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 List of samples.

Table S2 Groups sampled.

Table S3 Microsatellite primer sets.

Table S4 Primers for all 36 SNP assays.

Table S5 Summary of population structure analyses for mtDNA, microsatellites, SNPs and combined nuclear markers (nDNA), and sensitivity analyses for linked SNP loci.

Table S6 Variable sites among all known mtDNA sperm whale haplotypes globally and GenBank accession numbers.

Table S7 Allele frequencies for each microsatellite locus, by strata.

Table S8 Allele frequencies for each SNP locus, by strata. Haplotype inference based on 'three-strata' phase data set.

Table S9 Pairwise divergence between strata comprised of samples from females and young.

Table S10 Pairwise divergence between Gulf of Alaska males and the three low latitude strata containing females.

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