



Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales

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ABSTRACT: We quantified the relative influence of maternal fidelity to feeding grounds and natal fidelity to breeding grounds on the population structure of humpback whales *Megaptera novaeangliae* based on an ocean-wide survey of mitochondrial (mt) DNA diversity in the North Pacific. For 2193 biopsy samples collected from whales in 10 feeding regions and 8 breeding regions during the winter and summer of 2004 to 2006, we first used microsatellite genotyping (average, 9.5 loci) to identify replicate samples. From sequences of the mtDNA control region (500 bp) we identified 28 unique haplotypes from 30 variable sites. Haplotype frequencies differed markedly among feeding regions (overall $F_{ST} = 0.121$, $\Phi_{ST} = 0.178$, $p < 0.0001$), supporting previous evidence of strong maternal fidelity. Haplotype frequencies also differed markedly among breeding regions (overall $F_{ST} = 0.093$, $\Phi_{ST} = 0.106$, $p < 0.0001$), providing evidence of strong natal fidelity. Although sex-biased dispersal was not evident, differentiation of microsatellite allele frequencies was weak compared to differentiation of mtDNA haplotypes, suggesting male-biased gene flow. Feeding and breeding regions showed significant differences in haplotype frequencies, even for regions known to be strongly connected by patterns of individual migration. Thus, the influence of migratory fidelity seems to operate somewhat independently on feeding and breeding grounds over an evolutionary time scale. This results in a complex population structure and the potential to define multiple units to conserve in either seasonal habitat.

KEY WORDS: mtDNA · Microsatellite genotypes · Migration · Genetic management units

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INTRODUCTION

The influence of maternal fidelity on migratory destinations has been described in several species of marine megafauna, based on the movement of naturally marked or tagged individuals and the geographic distribution of variation in the maternally inherited mitochondrial (mt) genome. While the migratory return of an individual to a seasonal habitat reflects the stability of fidelity over a lifespan, the distribution of mtDNA diversity reflects the stability of maternal fidelity over generations on an evolutionary time scale. In some species of migratory whales, differences in the frequencies of mtDNA haplotypes among seasonal habitats are thought to arise from the persistence of maternal traditions learned during the first year of life. The loss of maternal traditions in some populations of whales has been attributed to intensive hunting, slowing the recovery of whales in regions of their former distribution (Whitehead et al. 2004, Clapham et al. 2008, Carroll et al. 2011).

Given the challenges of collecting representative samples from migratory whales, it has not been possible previously to adequately compare the relative influence of maternal fidelity to feeding habitat and natal fidelity to breeding habitat. The latter is more properly referred to as philopatry or natal philopatry—the faithful return of an individual to the site of its birth (Pearce 2007). In many species of marine megafauna, including pinnipeds, sea turtles, sharks and anadromous salmon, natal philopatry or natal homing is strongly linked to the breeding habitat. During the remainder of the life cycle, individuals are relatively nomadic, dispersing widely and mixing with individuals from other natal breeding grounds (e.g. sea turtles; Bowen & Karl 2007 and salmon; Quinn 2005). In whales, however, populations are structured by annual long-distance migration to both seasonal habitats, traditionally referred to as ‘breeding grounds’ and ‘feeding grounds’. A distinction between maternal fidelity and natal fidelity thus requires a comprehensive survey of mtDNA differentiation in multiple, discrete population segments during both the winter breeding season and the summer feeding season. To date, however, descriptions of mtDNA differentiation in humpback whales *Megaptera novaeangliae* have been restricted largely to comparisons among feeding grounds (e.g. Palsbøll et al. 1995) or among breeding grounds (e.g. Olavarria et al. 2007). Comparisons between seasonal habitats have involved only limited numbers of regional

samples or lower-resolution markers (e.g. Baker et al. 1998).

Humpback whales in the North Pacific Ocean present a complex structure of migration suitable for a comparison of the relative strengths of fidelity to feeding and breeding grounds. During spring, summer and autumn, humpback whales aggregate in relatively discontinuous feeding grounds along the rim of the North Pacific from central California in the east, to the Kamchatka Peninsula in the west (Johnson & Wolman 1984). During winter months, humpbacks migrate to mate and give birth in near-tropical breeding grounds of the eastern, central and western North Pacific. Although hunting severely reduced the abundance of humpback whales and might have altered their historical range, winter concentrations are now found primarily in 5 geographically isolated regions: the Pacific coast of Mexico, the off-shore islands of Mexico (Archipelago Revillagigedo), the main or leeward islands of Hawaii, the Ogasawara or Bonin Islands southeast of Japan, and the Ryukyu Islands (e.g. Okinawa) southwest of Japan (Johnson & Wolman 1984). More recently, smaller wintering concentrations have been found along the Central American coast in the east (Steiger & Calambokidis 1991) and the Philippines in the west (Acebes et al. 2007). Observations of individual movement and the distribution of mtDNA have provided strong support for fidelity to feeding grounds, particularly in California and Alaska, but evidence of natal philopatry (to breeding grounds) has been more challenging. The occasional long-range movement between wintering grounds (e.g. Japan to Hawaii; Darling & Cerchio 1993), the synchronous exchange of song (Cerchio et al. 2001) and contrasting patterns of variation in mtDNA and nuclear (nu)DNA (Palumbi & Baker 1994) provide evidence counter to natal fidelity.

Previous limitations on the sampling of an oceanic population have been addressed in surveys of the research program Structure of Populations, Levels of Abundance and Status of Humpbacks (SPLASH). This international collaboration was initiated to estimate the current abundance of North Pacific humpback whales, using capture-recapture analyses of photo-identification records, and to describe the seasonal structure of this population, using genetic analyses of skin biopsy samples (Calambokidis et al. 2008). Identification photographs and genetic samples were collected on all known winter breeding regions during 3 seasons (2004, 2005 and 2006) and all known summer feeding regions during 2 seasons (2004 and 2005). From more than 18 000 photo-

identification records of nearly 8000 individuals, the oceanic population was estimated to number more than 21 000 in 2006 (Barlow et al. 2011). This represents a remarkable recovery from the estimate of less than 1000 individuals thought to have survived at the time of protection from commercial hunting in 1966 (Johnson & Wolman 1984).

Here, we describe the structure of mtDNA diversity in the North Pacific humpback whale using a representative subset of 2193 skin samples from 10 feeding and 8 breeding grounds collected during the SPLASH program. To avoid bias from replicate sampling (Taylor & Chivers 1997), we used microsatellite genotyping to identify individual whales and remove regional replicates. Given the geographic isolation of breeding grounds and discontinuities in the concentration of sampling on most feeding grounds, we relied on the *a priori* regional divisions considered in previous estimates of abundance and migratory interchange for photo-identification records (Calambokidis et al. 2008, Barlow et al. 2011). We predicted that the distribution of mtDNA diversity, in the form of distinct haplotypes, should reflect the influence of maternally directed fidelity to both feeding and breeding habitats (Baker et al. 1990). Alternatively, the nearly unlimited mobility and long lifespan of humpback whales could result in sufficient interchange to counter the effect of genetic drift, resulting in little or no differentiation in haplotype frequencies. Given the large number of samples and the *a priori* geographic divisions, we used tests of differentiation to reject the null hypothesis of random assortment or panmixia, and F_{ST} or related indices (Meirans & Hedrick 2011) as a measure of genetic differences (i.e. the effect size; Taylor & Dizon 1996). We also tested for sex-biased dispersal by comparing estimates of mtDNA differentiation for males and females, under the expectation that F_{ST} will be higher in the philopatric sex (Goudet et al. 2002). The previously reported pattern of male-biased gene flow among breeding grounds was evaluated by comparing estimates derived from F_{ST} values for the maternally inherited mtDNA with those from the biparentally inherited microsatellite alleles (Palumbi & Baker 1994, Baker et al. 1998). We interpret our results in light of the complex structure of migratory interchange of humpback whales, providing new information for defining units to conserve or distinct population segments within the oceanic population (Taylor 2005, Waples 2006). These results have important implications for the status review of humpback whales currently ongoing under provisions of the US Endangered Species Act.

MATERIALS AND METHODS

Sample collection and archiving

Skin samples were collected from humpback whales on 10 feeding grounds and 8 breeding grounds of the North Pacific between 2004 and 2006 under the auspices of the program SPLASH (Calambokidis et al. 2008). Biopsy samples were collected with a small stainless-steel dart deployed from a crossbow (Lambertsen 1987) or modified veterinary capture rifle (Calambokidis et al. 2008). In some regions, sloughed skin was collected following high-energy behavior or from the paths of whales, using a small scoop net. All samples were archived at the Southwest Fisheries Science Center (SWFSC) in La Jolla, CA, and assigned accession numbers for cross-reference with the primary field records and photo-identification records archived with Cascadia Research.

A subset of the total SPLASH samples was chosen for analysis of mtDNA diversity and population differentiation ($n = 2193$; Table 1) with the objective of providing robust tests of differentiation by including a minimum of 100 individuals for each region (Taylor & Dizon 1996). We gave priority to the analysis of samples from 2004, because of the more extensive and systematic sampling of the feeding grounds in that year (Barlow et al. 2011) and because of logistical constraints in the availability of samples from later years. In 9 regions, however, we used all available samples to achieve or exceed this minimum (Table 1). The geographical divisions of feeding and breeding grounds were similar to those reported in Barlow et al. (2011) but followed Calambokidis et al. (2008) in grouping samples from the Commander Islands with the Kamchatka Peninsula to form a Russian feeding region, and subdividing the western breeding grounds into 3 regions: Ogasawara, Okinawa and the Philippines. The locations of sample collections and the approximate boundaries of the 18 geographic divisions are shown in Fig. 2a in 'Results' and Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m494p291_supp.pdf.

DNA extraction, quantification and sex identification

Total genomic DNA was extracted at the SWFSC and an aliquot was transferred to the Cetacean Conservation Genetic Laboratory (CCGL) of the Marine Mammal Institute, Oregon State University. At the CCGL, total genomic DNA was quantified using

Table 1. *Megaptera novaeangliae*. Summary of samples available for genetic analysis of humpback whales in the North Pacific. Regional divisions followed those used in SPLASH (Calambokidis et al. 2008). Abbreviations: N_{QC} is the number of samples after quality control (see text); N_I is the number of individuals in each region; N_{I+hap} is the number of individuals with a mitochondrial (mt) DNA control region sequence; h is haplotype diversity; M:F is the ratio of males to females and p is the significance of the binomial exact test for an expected 1:1 sex ratio (values in **bold** are significant at $p < 0.05$). *Italics* indicate low sample sizes. *Regions for which all available samples were used

Region (abbreviation)	All	N_{QC}	N_I	N_{I+hap}	h	M:F	p
Russia (RUS)*	82	79	72	70	0.831	0.69:1	0.154
<i>Western Aleutians (WAL)*</i>	<i>9</i>	<i>8</i>	<i>8</i>	<i>8</i>	<i>0.964</i>	<i>1.66:1</i>	–
Bering Sea (BER)	137	129	119	114	0.798	0.69:1	0.062
Eastern Aleutians (EAL)*	37	37	36	36	0.848	2.09:1	0.058
Western Gulf of Alaska (WGOA)	117	109	98	96	0.845	0.67:1	0.076
Northern Gulf of Alaska (NGOA)	253	247	237	233	0.780	1.16:1	0.292
Southeast Alaska (SEA)	214	199	186	183	0.472	1.09:1	0.606
Northern British Columbia (NBC)	123	122	108	104	0.550	1.32:1	0.218
Southern British Columbia-Washington (SBC/WA)*	57	57	51	51	0.831	1.38:1	0.332
California-Oregon (CA/OR)*	136	134	125	123	0.827	1.25:1	0.243
Total feeding regions	1165	1121	1040	1018	0.810	1.04:1	0.508
<i>Philippines (PHI)*</i>	<i>13</i>	<i>13</i>	<i>13</i>	<i>13</i>	<i>0.628</i>	<i>1.17:1</i>	–
Okinawa (OK)*	96	90	72	72	0.655	2.24:1	0.002
Ogasawara (OG)*	241	222	163	159	0.865	1.92:1	0.001
Hawaii (HI)	278	245	231	227	0.718	2.67:1	0.001
Mexico-Revillagigedo (MX-AR)	145	141	115	106	0.860	1.67:1	0.010
Mexico-Baja California (MX-BC)	137	137	120	110	0.887	2.50:1	0.001
Mexico-Mainland (MX-ML)	75	75	62	62	0.894	2.16:1	0.006
Central America (CENAM)*	43	41	39	36	0.744	2.92:1	0.073
Total breeding regions	1028	964	815	785	0.866	2.17:1	0.001
Total by regions	2193	2085	1855	1803	0.842	1.42:1	0.001
Total number of individuals for all regions	1805						

pico-green fluorescence and normalized to $5 \text{ ng } \mu\text{l}^{-1}$ before amplification, where possible. The sex of each sampled whale was identified by multiplex amplification of the male-specific *Sry* gene with *ZFY/ZFX* as a positive control (Gilson et al. 1998).

mtDNA sequencing

An approximately 800 base pair (bp) section of the mtDNA control region was amplified using the primers light-strand tPro-whale Dlp-1.5 and heavy strand Dlp-8G as described in (Garrigue et al. 2004). Unincorporated nucleotides and primers were removed from the amplified product using shrimp alkaline phosphatase (SAP) and exonuclease I (Ex). Purified products were sequenced with BigDye vs3.1 and run on an ABI 3730xl (Applied Biosystems). Sequences of the mtDNA control region were aligned, manually edited, and haplotypes identified using the software

SEQUENCHER (Gene Codes). Haplotypes were defined from a 500 bp segment of sequences extending across the 2 shorter, and partially overlapping, fragments used in past analyses (Baker & Medrano-Gonzalez 2002). Following recommended guidelines for quality control (Morin et al. 2010), sequence quality was calculated in SEQUENCHER from the ABI base scores using a cut off equivalent to a PHRED score of >30 (i.e. an error rate of less than 1 in 1000; Ewing & Green 1998). Variable sites in all samples were visually assessed to confirm haplotype identity. One haplotype was represented by only 1 sample and this was confirmed by sequencing in both directions from independent PCR products. A small number of individuals ($n = 18$) showed apparent heteroplasmy (i.e. the presence of a secondary peak at greater than 30% of the primary peak), confounding the resolution of a single haplotype. Rather than attempt to define multiple haplotypes for individual samples, these were treated as missing values in subsequent analyses.

Table 2. *Megaptera novaeangliae*. Summary of microsatellite loci used for individual identification of humpback whales in the North Pacific. The observed heterozygosity (H_o) and probability of identity (P_{ID}) were calculated with the program GENALEX (Peakall & Smouse 2006). The multi-locus probability of identity is shown below the average single-locus value

Locus	N*	Source	Label	[Mg] (mM)	No. of repeats	Size range (bp)	No. of alleles	H_o	P_{ID}
Ev14	1835	Valsecchi & Amos (1996)	VIC	2.5	2	129–141	7	0.609	0.177
Ev37	1830	Valsecchi & Amos (1996)	NED	3.5	2	192–220	15	0.867	0.024
Ev96	1833	Valsecchi & Amos (1996)	FAM	1.5	2	143–171	14	0.751	0.079
GATA417	1797	Palsbøll et al. (1997)	FAM	2.5	4	183–274	18	0.855	0.028
GATA28	1844	Palsbøll et al. (1997)	NED	2.5	4	143–191	9	0.407	0.353
GT211	1844	Bérubé et al. (2000)	FAM	2.5	2	100–118	9	0.758	0.076
GT23	1807	Bérubé et al. (2000)	VIC	2.5	2	109–121	7	0.699	0.111
GT575	1800	Bérubé et al. (2000)	FAM	1.5	2	137–165	13	0.794	0.056
rw4-10	1844	Waldick et al. (1999)	VIC	3	2	192–208	9	0.677	0.130
rw48	1838	Waldick et al. (1999)	NED	2.5	2	112–122	6	0.695	0.117
Average	1827.2						10.7	0.711	0.115
									2.4×10^{-11}

*Number of individuals genotyped at each locus, including between-region re-matches

Microsatellite genotyping

Samples were amplified for a total of 10 previously published microsatellite loci (Table 2). Amplifications were carried out in a final volume of 10 μ l at the following concentrations: 1 \times reaction buffer, 1.5 to 4 mM MgCl₂, 0.4 μ M each primer, 0.2 mM dNTP's, 0.5 U Platinum *Taq* (Invitrogen) and approximately 5 ng DNA. Microsatellite primers were fluorescently labeled (Table 2) and co-loaded in sets of non-overlapping loci on an ABI 3730xl sequencer (Applied Biosystems) with formamide and 500 LIZTM size standard (Applied Biosystems). Alleles were sized and binned with the software program GENEMAPPER v3.7 (Applied Biosystems). This program also assesses the quality of each allele based on several criteria (e.g. peak height, peak shape, bin fit) and assigns a quality score (QS) for each sample. The peaks for all alleles were visually assessed and the automated bins were manually checked. Following recommended quality control guidelines from Morin et al. (2010), a subset of samples were re-run at random, and re-samples of known individuals provided additional replicates. The total number of amplified loci was considered an additional quality control threshold. Samples with fewer than 8 microsatellite loci were excluded from further analysis. The program MICROCHECKER was used to investigate the presence of null alleles and systematic error in allele binning due to stutter or large allele dropout (van Oosterhout et al. 2004). Replicate genotypes were identified with the program CERVUS (Kalinowski et al. 2007). To avoid the potential for false exclusion due to dropout or other genotyping errors (Waits & Leberg 2000,

Waits et al. 2001), we used an initial relaxed criterion that allowed for mismatches at up to 4 loci. The electropherograms of the mismatching loci were then reviewed and either corrected (based on this visual inspection) or repeated. Because of some incomplete genotypes, we required a minimum overlap of 8 loci to identify replicate samples of individual. Where a pair-wise comparison did not meet this minimum overlap, the missing loci for the limiting genotype were repeated or the sample was removed from the data set. A per-allele error rate was calculated from replicate genotypes identified with the initial relaxed matching and subsequent review of near matches (Pompanon et al. 2005).

Statistical analyses

The program ARLEQUIN (Excoffier & Lischer 2010) was used to infer a network of mtDNA haplotypes, to calculate haplotype diversity and indices of regional differentiation (F_{ST} and Φ_{ST}). The significance of regional differentiation was tested with 10 000 random permutations using an Analysis of Molecular Variance (AMOVA). The expected probability of identity (P_{ID}) for each locus was calculated for each regional sample, after removal of replicate samples, using GENALEX (Peakall & Smouse 2006). All genotype matches were checked for consistency with sex and mtDNA haplotypes.

The significance of deviation from Hardy-Weinberg expectations and regional differentiation in microsatellite allele frequencies (i.e. F_{ST}) was tested with the program GENEPOP (Rousset 2008). Supple-

mentary calculations of F_{ST} for mtDNA and adjusted F'_{ST} for microsatellites (Meirmans & Hedrick 2011), as well as Mantel tests for the correlations of the pair-wise F_{ST} values, were calculated in GENODIVE (Meirmans & Van Tienderen 2004). Tests for sex-biased dispersal were conducted for mtDNA and microsatellites using the program FSTAT (Goudet et al. 2002). Maternal gene flow (Nm_f) was calculated from mtDNA F_{ST} using the relationship:

$$Nm_f = \frac{1}{2}(1/F_{ST} - 1)$$

assuming Wright's island model (Wright 1951). Nuclear gene flow, Nm_{f+m} , was calculated from a combination of microsatellite F_{ST} and F'_{ST} using Eq. 6 in Meirmans & Hedrick (2011). To investigate the potential for inferring reproductive units from the combined feeding and breeding ground samples in the absence of the *a priori* geographical divisions, we used the Bayesian clustering program STRUCTURE v. 2.3 (Pritchard et al. 2000). We evaluated a range of potential populations from $K = 1$ to 6 using the admixture and correlated frequency model with 1 000 000 burn-ins and 10 000 000 runs and no location prior.

To protect against Type I error in our tests of differentiation, we relied on first demonstrating the significance of the overall differences among breeding grounds and among feeding grounds. These tests reflected our primary hypotheses of natal philopatry and maternal fidelity, respectively. For the many pair-wise comparisons of the 18 regions, we did not consider it appropriate to implement a Bonferroni correction, given the known conservative bias of this adjustment (Nakagawa 2004, Narum 2006). Instead, we reported the unadjusted p-values and controlled for the proportion of falsely rejected hypotheses (i.e. Type I error) in the pair-wise comparisons by using the false discovery rate, as formulated by Benjamini & Hochberg (1995) and reported by Narum (2006). By reporting both the unadjusted p-values and the critical value for the false discovery rate, we provided for interpretations of differences among the pair-wise comparisons in a management context, where loss of power is a greater concern than Type I error (Taylor et al. 1997).

RESULTS

Genotype matching and sex ratios

Of the 2193 total samples chosen for the initial analysis, 2085 met the quality control threshold by

genotyping at a minimum of 8 loci (Table 1). The 10 microsatellite loci were all found to be moderately variable with 6 to 18 alleles per locus (Table 2). The program MICROCHECKER found no evidence of large-allele dropout or error due to stutter (only one of the 180 tests from 18 populations and 10 loci, using the 95 % threshold). Evidence for some null alleles was found in 17 of the 180 tests (at the 95 % threshold). However, most of these positive tests were in regional samples thought to represent a mix of breeding stocks (e.g. Ogasawara, see below), suggesting a potential for a Wahlund effect. Given that our primary purpose was the identification of replicate samples, we considered it appropriate to retain these loci (Carlsson 2008). From the initial relaxed matching and review of near-matches, 227 individuals were found to have one or more replicate samples (e.g. within- or between-region recaptures). From these capture-recapture events, we calculated an initial error rate of 1.6 % per allele, most of which was attributable to allelic dropout (Pompanon et al. 2005). This rate was comparable to other studies using unintentional replicates to estimate genotyping error (Hess et al. 2012) but higher than some studies of whales based only on biopsy samples (Carroll et al. 2011). After the detection and correction of initial errors by repeating the genotypes or reviewing original electropherograms, the final error rate was likely to be considerably less.

With the minimum of 8 loci available for each sample, the P_{ID} was less than 1×10^{-6} , regardless of population of origin or the specific combination of loci. Given this low value, we considered that matching genotypes represented multiple samples of the same individual and unique genotypes represented unique individuals. From these 2085 samples, genotype matching with the program CERVUS resolved 1855 regional individuals (unique within a region). Within-region matches were considered to be replicates and were removed from further genetic analysis (see Table S1 in the supplement at www.int-res.com/articles/suppl.m494p291_supp.pdf, shown in the diagonals).

From the total of 1855 regional individuals identified by unique genotypes, there were 50 matches of 49 individuals sampled in more than one region (48 in 2 regions and 1 in 3 regions). These between-region matches (i.e. recaptures) were retained for analysis of regional population differentiation (Table S1 in the supplement, shown below the diagonals). A small number of recaptures showed within-season movement between adjacent feeding grounds ($n = 4$) and between adjacent breeding grounds ($n = 14$), particu-

larly between the Mexican Mainland and Baja California (9 of the 14). The remaining recaptures (34 recaptures of 33 individuals) showed movement between breeding and feeding grounds. These migratory connections were generally consistent with those published previously or reported in the photo-identification analysis of SPLASH (Calambokidis et al. 2008, Barlow et al. 2011).

The overall sex ratio of samples from the breeding grounds showed a significant male bias (2.17:1, $n = 782$, $p < 0.001$; Table 1) similar to that reported previously from genetic sampling of breeding grounds (Baker et al. 1998) or migratory corridors (Brown et al. 1995). The overall sex ratio of samples from the feeding grounds was close to unity overall (1.04:1, $n = 1004$, $p = 0.508$) but showed a significant male bias in the Eastern Aleutian Islands and a significant female bias in the Bering Sea (Table 1).

mtDNA diversity and differentiation

Of the 1855 samples of regional individuals, 1803 yielded high-quality sequences of the mtDNA control region (Table 1). In the 500 bp consensus length, we identified 30 variable sites resolving 28 unique haplotypes (Fig. 1). A parsimony network reconstructed the 2 primary clades of haplotypes described previously from a worldwide survey of mtDNA diversity (Baker et al. 1993): the AE clade, largely restricted to

the North Pacific, and the CDF clade, thought to have originated from historical interchange with populations from the Southern Hemisphere (Fig. 1). Four haplotypes, A+, A-, E1 and F2, accounted for 70% of all samples on both feeding grounds and breeding grounds (Fig. 1). Haplotype diversity was similar in feeding and breeding grounds overall, but showed substantial differences among regions. Haplotype diversity was notably low in southeastern Alaska ($h = 0.47$) and highest in the Mexican Mainland and Baja California ($h = 0.89$). Sequences representing each of the 28 haplotypes have been submitted to GenBank (KF477244-KF477271) and information on regional frequencies of haplotypes has been submitted to the Dryad Digital Repository (<http://dx.doi.org/10.5061/dryad.708j7>).

There were striking differences in the regional frequencies of mtDNA haplotypes (Fig. 2b). Tests of differentiation showed highly significant differences among feeding regions (overall $F_{ST} = 0.121$, $\Phi_{ST} = 0.178$, $p < 0.0001$) and among breeding grounds (overall $F_{ST} = 0.093$, $\Phi_{ST} = 0.106$, $p < 0.0001$) (Table 3). Pairwise comparison differed significantly ($p < 0.05$, Table 4) at 18 of the 21 pairs of breeding grounds (excluding the Philippines) and 32 of 36 pairs of feeding grounds (excluding Western Aleutians). The number of significant comparisons was not affected by the adjustment for the false discovery rate (critical value, $p < 0.0375$). Among feeding regions, Russia, southeastern Alaska and California/Oregon were notable for particularly high levels of differentiation from each other (up to $F_{ST} = 0.343$). Among breeding regions, Okinawa and Central America were notable for a particularly high level of differentiation from each other ($\Phi_{ST} = 0.461$) and from most other breeding grounds (Table S2 in the supplement).

A comparison between feeding and breeding regions also showed a large number of significant differences; 54 of 63 comparisons at $p < 0.05$, or 53 of 63 after adjustment for the false discovery rate. These differences were significant even for regions known to be strongly connected by patterns of individual migration (e.g. by photo-identification, Calambokidis et al. 2008). For example, Okinawa differed significantly from all feeding regions, including Russia. Southeastern Alaska also differed from all breeding grounds, including Hawaii. In other cases, similarities of haplotypes frequencies, as indicated by small values of F_{ST} , were consistent with known migratory connections. For example, Western Gulf of Alaska showed no significant differentiation from the 3 Mexican breeding regions. Central

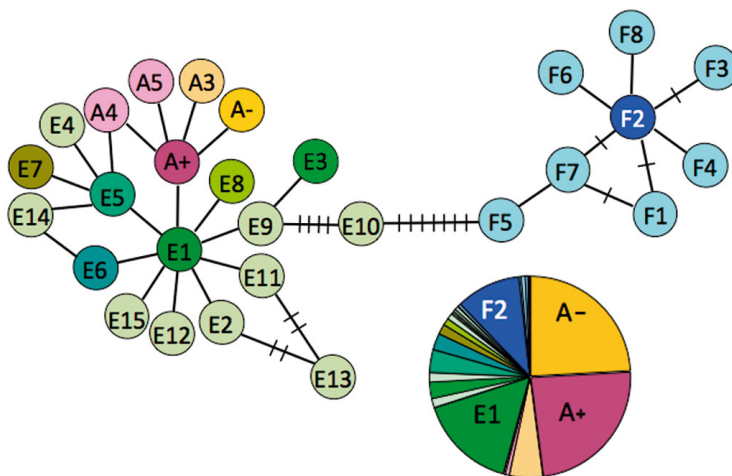


Fig. 1. *Megaptera novaeangliae*. A parsimony network showing connections between the 28 described mitochondrial (mt) DNA haplotypes for humpback whales in the North Pacific and a pie chart of overall frequencies for all regional samples combined. Colors relate to the 3 haplogroup designations from previous publications (e.g. Baker et al. 1998): A-types are shaded maroon and yellow, E-types are shaded green, F-types are shaded blue. Each line indicates one base difference between haplotypes

Table 3. *Megaptera novaeangliae*. Differentiation of mitochondrial (mt) DNA and microsatellite loci among breeding grounds, among feeding grounds and among all regions for humpback whales in the North Pacific. Conventional F_{ST} for mtDNA and microsatellites and adjusted F'_{ST} for microsatellites are calculated for all samples and for each sex. F'_{ST} and bootstrap confidence limits (CL) for microsatellites were calculated with the program GENODIVE (Meirmans & Van Tienderen 2004). Differences between sex-specific F_{ST} were tested using the program FSTAT (Goudet et al. 2002). Nm_{f+mt} for microsatellites was calculated using Eq. (6) in Meirmans & Hedrick (2011)

Habitat (n = regions)	Sex	mtDNA			Microsatellite loci			
		F_{ST} (Φ_{ST})	p	Nm_f	F_{ST} (95 % CL)	F'_{ST}	p	Nm_{f+mt} (95 % CL)
Breeding + feeding (n = 18)	Male + female	0.109 (0.138)	<0.001	4.1	0.0043 (0.0028–0.0054)	0.0155	<0.001	57 (46–87)
	Male	0.103	<0.001		0.0047	0.0174	<0.001	
	Female	0.116	<0.001		0.0034	0.0126	<0.001	
Breeding (n = 7) ^a	Male + female	0.093 (0.106)	<0.001	4.9	0.0061 (0.0039–0.0076)	0.0209	<0.001	40 (33–64)
	Male	0.090	<0.001		0.0067	0.0247	<0.001	
	Female	0.098	<0.001		0.0039	0.0145	<0.004	
	Sex difference	–0.008	0.420		0.0028		0.181	
Feeding (n = 9) ^b	Male + female	0.121 (0.178)	<0.001	3.6	0.0034 (0.0026 – 0.0043)	0.0128	<0.001	73 (58–96)
	Male	0.114	<0.001		0.0041	0.0151	<0.001	
	Female	0.124	<0.001		0.0034	0.0125	<0.001	
	Sex difference	–0.010	0.296		0.0007		0.715	

^aExcluding Philippines; ^bexcluding Western Aleutians

grounds (overall $F_{ST} = 0.0061$, $p < 0.001$). After application of an adjustment for diversity (Hedrick 2005), the effect size increased to $F'_{ST} = 0.0128$ and $F'_{ST} = 0.0214$ for feeding and breeding grounds, respectively. Pair-wise comparisons differed significantly ($p < 0.05$) at 18 of the 21 pairs of breeding grounds (excluding the Philippines) and 31 of 36 pairs of feeding grounds (excluding Western Aleutians). After adjustment for the false discovery rate (critical value, $p < 0.0343$), this was reduced to 17 and 30, for breeding and feeding grounds, respectively. A comparison between feeding and breeding grounds also showed a large number of significant differences; 49 of 63 pair-wise comparisons at $p < 0.05$, or 46 after adjustment for the false discovery rate. As with mtDNA, these differences were significant even for regions known to be strongly connected by patterns of individual migration (Tables S3 & S4 in the supplement).

Exploratory analyses of microsatellite genotypes with the program STRUCTURE showed little evidence of detectable clusters in the absence of *a priori* geographic divisions. For a range of $K = 1$ to 6, the highest likelihood was for $K = 1$ (i.e. one population). For $K > 1$, the ΔK method of Evanno et al. (2005) suggested $K = 2$ provided the best fit for the data. However, on inspection of the membership coefficients, all individuals were admixed and showed nearly equal assignment to each of the K clusters (Fig. S1 in the supplement). These results are consistent with

the known limits of STRUCTURE to detect population structure where differentiation of microsatellite allele frequencies is weak and the number of loci is modest (Latch et al. 2006, Waples & Gaggiotti 2006).

Despite the weak effect size observed for microsatellite loci, however, a Mantel test of the pair-wise matrices of F_{ST} values for microsatellite and mtDNA showed a significant overall correlation ($r = 0.609$, $p < 0.001$; Fig. S2). This correlation was stronger for the 7 breeding grounds ($r = 0.659$, $p < 0.01$), but was also significant among the 9 feeding grounds ($r = 0.553$, $p < 0.001$).

Sex-biased dispersal and gene flow

We tested for sex-biased dispersal by comparing sex-specific estimates of mtDNA and microsatellite F_{ST} on breeding grounds and feeding grounds, using the permutation procedure in FSTAT. Although mtDNA F_{ST} values were slightly higher for females and microsatellites F_{ST} values were slightly higher for males, these differences were not significant. Despite the absence of apparent sex-biased dispersal, a comparison of conventional gene flow estimates (Nm) showed large differences in the values derived from the biparentally inherited microsatellites and the maternally inherited mtDNA (Table 3). To test the differences observed among breeding

Table 4. *Megaptera novaeangliae*. Pair-wise F_{ST} values for mitochondrial (mt) DNA haplotypes of North Pacific humpback whales for (a) feeding grounds, (b) breeding and feeding grounds and (c) breeding grounds. *Significant at <0.05, **significant at <0.01 and ***significant at <0.001, unadjusted for multiple comparisons. **Bold** values were significant after adjustment for the false discover rate. F_{ST} indices and significance of pair-wise differences (10 000 permutations) were calculated with the program ARLEQUIN (Excoffier & Lischer 2010). Rows and columns in *italics* indicate low sample sizes for comparisons involving Western Aleutians (WAL) or Philippines (PPH)

(a)	RUS	WAL	BER	EAL	WGOA	NGOA	SEA	NBC	SBC/WA	CA/OR
RUS	–									
WAL	<i>0.000</i>	–								
BER	0.081***	<i>0.000</i>	–							
EAL	0.069***	<i>0.000</i>	0.000	–						
WGOA	0.033**	<i>0.000</i>	0.023**	0.003	–					
NGOA	0.113***	<i>0.017</i>	0.039***	0.011	0.027**	–				
SEA	0.317***	0.282**	0.245***	0.224***	0.198***	0.095***	–			
NBC	0.252***	0.206**	0.175***	0.153***	0.139***	0.057***	0.003	–		
SBC/WA	0.076***	<i>0.014</i>	0.049***	0.034*	0.020*	0.035**	0.191***	0.127***	–	
CA/OR	0.081***	<i>0.001</i>	0.082***	0.053***	0.069***	0.124***	0.343***	0.283***	0.107***	–
(b)	RUS	WAL	BER	EAL	WGOA	NGOA	SEA	NBC	SBC/WA	CA/OR
PHI	<i>0.036</i>	<i>0.050</i>	0.165***	0.159***	0.094**	0.218***	0.474***	0.406***	0.166***	0.123***
OK	0.035**	<i>0.050</i>	0.166***	0.173***	0.100***	0.217***	0.434***	0.373***	0.169***	0.152***
OG	0.016*	<i>0.000</i>	0.064***	0.052***	0.031***	0.097***	0.269***	0.211***	0.063***	0.081***
HI	0.158***	<i>0.068</i>	0.063***	0.036*	0.058***	0.004	0.072***	0.034***	0.057***	0.163***
MX-AR	0.034**	<i>0.000</i>	0.011*	0.000	0.000	0.040***	0.233***	0.169***	0.032**	0.066***
MX-BC	0.028***	<i>0.000</i>	0.035***	0.007	0.000	0.039***	0.214***	0.157***	0.023*	0.047***
MX-ML	0.035**	<i>0.000</i>	0.013	0.002	0.011	0.051***	0.265***	0.191***	0.026*	0.045***
CENAM	0.076***	<i>0.022</i>	0.132***	0.104***	0.092***	0.179***	0.433***	0.367***	0.154***	0.006
(c)	PHI	OK	OG	HI	MX-AR	MX-BC	MX-ML	CENAM		
PHI	–									
OK	<i>0.000</i>	–								
OG	0.069**	0.059***	–							
HI	0.276***	0.268***	0.130***	–						
MX-AR	0.103**	0.040***	0.031***	0.075***	–					
MX-BC	0.096**	0.104***	0.032***	0.075***	0.002	–				
MX-ML	0.117***	0.120***	0.020**	0.081***	0.004	0.007	–			
CENAM	0.078*	0.117***	0.087***	0.228***	0.096***	0.071***	0.081***	–		

Table 5. *Megaptera novaeangliae*. Temporal comparison of mitochondrial (mt) DNA haplotype frequencies for humpback whales in the Central California/Oregon and southeast Alaska feeding grounds using SPLASH samples from 2004 and pre-SPLASH samples from 1987 to 1989. Differences in frequencies were tested with the program GENEPOP (Rousset 2008)

Region	Haplotype code																	Total	
	A-	A+	A4	E1	E5	E6	E2	E3	E10	E13	F1	F2	F6	F3	F4	F8	A3		E4
Southeast Alaska (p = 0.21)																			
SPLASH (2004)	123	51	1	0	0	0	7	0	0	0	0	1	0	0	0	0	0	0	183
pre-SPLASH (1987–88)	38	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54
California/Oregon (p = 0.33)																			
SPLASH (2004)	2	8	0	24	2	3	0	2	3	5	5	43	3	4	1	1	5	10	123
pre-SPLASH (1988–89)	0	4	0	11	2	3	0	1	2	2	2	17	3	2	0	0	0	0	49

grounds, we compared the point estimate of female migration calculated from the mtDNA F_{ST} to the estimates of male and female migration calculated from the 95% confidence limits of the microsatellite F_{ST} , based on bootstrapping loci in the program GENODIVE. Assuming Wright's island model (Wright 1951)

with an equal number of male and female migrants and with equal reproductive variance, the gene flow estimates from nuclear microsatellites should be twice that from mtDNA (Birky et al. 1983). Although it is likely that the assumptions of Wright's model are violated in North Pacific humpback whales, as in

most biological populations (Whitlock & McCauley 1999), our intent was not to measure the true rate of effective dispersal by males and females. Instead, we used the calculated values of Nm , which adjust for the different modes of inheritance (Ennos 1994), to test the null hypothesis that the estimates of F_{ST} from mtDNA and from microsatellites were consistent with equal gene flow by males and females. This was not the case. Instead, the estimate of $Nm_{f+m} = 32.0$ calculated from the upper 95% confidence limit of the microsatellite F_{ST} (i.e. the lower 95% confidence limit of nuclear gene flow) was several-fold greater than the estimate of $Nm_f = 4.9$ for mtDNA (Table 4). For the point estimates of mtDNA and microsatellite F_{ST} from the breeding grounds, we used the methods of Hedrick et al. (2013) to calculate male gene flow as $Nm_m = 36$, and the ratio of male to female gene flow as $m_m/m_f = 7.4$. Although we considered it meaningful to interpret this difference only for the breeding grounds, the influence of male-biased gene flow was also apparent in the comparison of mtDNA and microsatellite F_{ST} for feeding grounds and among all regions (Table 3).

DISCUSSION

Migratory fidelity

This first ocean-wide survey of mtDNA differentiation in humpback whales from the North Pacific has demonstrated the strong influence of maternal fidelity to feeding grounds, and natal philopatry to breeding grounds. The results are consistent with, but greatly extend, previous surveys of mtDNA diversity, which were limited by geographic sampling or sequence resolution (Baker et al. 1994, Medrano-Gonzalez et al. 1995, Baker et al. 1998, Witteveen et al. 2004). In particular, the extended sampling demonstrated that breeding grounds are strongly differentiated by mtDNA haplotype frequencies, although only weakly differentiated by microsatellite allele frequencies (see below). In this, humpback whales are similar to southern right whales (Patenaude et al. 2007, Carroll et al. 2011).

The influences of maternal fidelity and natal philopatry on genetic structure persist despite the obvious potential for individual humpback whales to migrate between alternate breeding grounds or feeding grounds. Although humpback whales can undertake considerable spatial exploration during a lifetime (Pomilla & Rosenbaum 2005, Robbins et al. 2011, Stevick et al. 2011), this exploration may be

temporary or unsuccessful for reproduction. Such temporary dispersal would reduce the influence on gene flow (Slatkin 1987), particularly female-mediated gene flow. The influence of maternal fidelity and natal philopatry have also persisted despite the rapid growth of the North Pacific population since protection from exploitation in 1966 (Johnson & Wolman 1984). Our temporal comparison of haplotype frequencies in southeastern Alaska and California/Oregon showed remarkable stability, despite the intervening period of 20 yr and probable differences in local distribution and sampling strategies in the different study periods. This represents approximately one generation for humpback whales and, given an approximately 7% rate of annual increase, a quadrupling of abundance (Barlow et al. 2011).

In showing both maternal fidelity to feeding grounds and natal philopatry to breeding grounds, we confirm that the pattern of migratory fidelity in humpback whales differs from most other marine megafauna. For sharks, sea turtles and anadromous fish, natal philopatry is presumably the result of behavioral imprinting on environmental cues or genetic encoding of a navigational map (Pardini et al. 2001, Lohmann et al. 2008, Jorgensen et al. 2010). For most whales, however, the close dependency of the calf with its mother during the first year of life and the first complete annual migration (e.g. Baker et al. 1987) provides a direct mechanism for a learned fidelity to both breeding and feeding habitat through maternal experience. This early maternal experience forms the basis for the cultural inheritance of migratory destination, which can be lost as a result of local exploitation (Clapham et al. 2008). Although it seems likely that optimal habitat can be rediscovered (e.g. Carroll et al. 2011), this rediscovery could require several generations, contributing to variability in the spatial recovery of some whale populations (Baker & Clapham 2004, Clapham et al. 2008).

Male-biased gene flow or male-biased dispersal?

Sex-biased dispersal is common in mammals, with males usually acting as the dispersing sex and females as the philopatric sex (Greenwood 1983). Among humpback whales in the North Pacific, however, we failed to find evidence of significant male-biased dispersal in the regional distribution of mtDNA haplotypes; i.e. sex-specific values of F_{ST} were similar among breeding grounds and among feeding grounds. This is consistent with photo-identification

records, where both males and females show strong fidelity in some habitats (e.g. southeastern Alaska), and where rare long-distance dispersal events (i.e. movement between alternate breeding grounds) have been reported for both sexes (Pomilla & Rosenbaum 2005, Stevick et al. 2011). It is also consistent with estimates of demographic closure for humpback whale breeding grounds in New Caledonia, based on gametic capture-recapture (Garrigue et al. 2004).

Despite the negative results for the tests of sex-biased dispersal, however, our results are consistent with previous reports of male-biased gene flow (Palumbi & Baker 1994, Baker et al. 1998), as inferred from the comparative magnitude of differentiation for mtDNA and the nuclear microsatellites. This apparent contradiction is explained most simply by the differences in the levels of interchange detectable by the 2 approaches. To detect male-biased dispersal using mtDNA frequencies, the levels of temporary demographic interchange (i.e. vagrancy) by males would probably have to be on the order of 10% (Goudet et al. 2002), even for regions with nearly fixed differences, e.g. California and southeastern Alaska. Given the size of these populations, this would require hundreds of annual vagrants. By comparison, the level of male-biased gene flow consistent with even the lower 95% confidence limits of the microsatellite F_{ST} from the breeding grounds (i.e. the upper 95% confidence limits of Nm_{f+m}), would be less than 100 vagrants per generation. Given the estimated generation time of 21.5 yr for humpback whales (Taylor et al. 2007), the equivalent number of annual vagrants would be too small to detect by differences in haplotype frequencies. Only very large-scale surveys of naturally marked or genotyped individuals would likely detect such low levels of interchange, as appears to be the case for SPLASH (Barlow et al. 2011).

Various hypotheses have been proposed for the evolution of sex-biased dispersal, including avoidance of inbreeding and local mate competition, and strategies for balancing these with the advantages of philopatry (Greenwood 1980, Dobson 1982, Shields 1982, Pusey 1987). It seems that male humpback whales could use a mixed-strategy over the course of a lifetime, involving occasional alteration among breeding grounds and gametic dispersal, without abandoning individual natal fidelity. Females could also visit alternate breeding grounds for mating opportunities, but unless this dispersal was permanent or contributed a female offspring through a migratory cycle, it would not contribute to mtDNA gene flow.

Complex structure of migratory populations

The population structure of North Pacific humpback whales is complex, as described previously from the documented migratory destinations of naturally marked individuals (Darling & Jurasz 1983, Darling & McSweeney 1985, Baker et al. 1986, Darling & Cerchio 1993, Calambokidis et al. 1996, Darling et al. 1996, Urbán et al. 2000, Calambokidis et al. 2001, Acebes et al. 2007). There are varying degrees of mixing between feeding and breeding grounds, as reflected in the regional return and interchange documented in the photo-identification records of SPLASH (Calambokidis et al. 2008, Barlow et al. 2011). For example, the southeastern Alaska feeding grounds shows a strong migratory connection only with the Hawaiian breeding grounds, but other whales from Hawaii migrate to the Northern Gulf of Alaska and other feeding grounds. Conversely, the Central American breeding grounds show a strong connection only with the California/Oregon feeding grounds, but other whales from California/Oregon migrate to the Mexican Mainland (Calambokidis et al. 2001). The Northern Gulf of Alaska shows migratory connections to breeding grounds from Asia to Mexico, e.g. Ogasawara, Hawaii, Mexico-Revillagigedo and Mexico-Mainland (Table S1).

These asymmetrical patterns of interchange between seasonal habitats are reflected, to some extent, by regional differences in frequencies of mtDNA haplotypes. As expected from the photo-identification records, southeastern Alaska differed significantly from all breeding grounds, including Hawaii, and all other feeding grounds, except Northern British Columbia. Hawaii differed from all other breeding grounds and all feeding grounds, except the Northern Gulf of Alaska and Eastern Aleutian Islands (and in the latter case, the lack of significant differentiation may be due to a small sample size). Central America showed no significant differentiation from California/Oregon, although California/Oregon differed significantly from Mexican Mainland, and from all other breeding and feeding grounds. As a consequence, southeastern Alaska appears to be a 'pure feeding stock' that mixes with others on the Hawaiian breeding grounds and Central America appears to be a 'pure breeding stock' than mixes with others on the California/Oregon feeding grounds.

The asymmetry of regional fidelity and mtDNA differentiation suggests that the influence of maternal fidelity operates somewhat independently on feeding and breeding grounds over an evolutionary time scale; i.e. individuals can share fidelity to one habitat

but not another. As a consequence, there are no simple divisions of the oceanic population into migratory subpopulations that remain isolated year-round. Neither is there an obvious allocation of breeding grounds and feeding grounds into 'pure' and 'mixed' stocks. A 'many-to-many' mixed-stock solution to this complex seasonal structure (Bolker et al. 2007) will require integration of the regional haplotype frequencies and estimated regional abundance, with records of interchange and return from photo-identification (e.g. SPLASH, Calambokidis et al. 2008).

Missing components of the population and stock uncertainty

Our results were consistent with the analysis of photo-identification records in suggesting that some components of the North Pacific population are underrepresented or missing in the SPLASH sampling (Calambokidis et al. 2008). Under-representation of some regions (or over-representation of others) was not surprising given the differences in sample size and regional abundance, particularly the large samples from the abundant feeding grounds of Northern Gulf of Alaska, southeastern Alaska and Northern British Columbia. These differences were not likely to influence the conclusions of the tests of differentiation presented here (except where sample sizes were very small, e.g. the Western Aleutians and Philippines). However, the potential for a missing component of the population is of greater concern for management purposes. This was most apparent in the western North Pacific, where we found significant differences in haplotype frequencies between Russian feeding grounds and both Okinawa and Ogasawara, despite the evidence of strong migratory connections between these seasonal habitats (Barlow et al. 2011). This suggested that a component of the western breeding grounds was missing or inadequately represented in the sampling of feeding grounds. The finding of 2 haplotypes (represented by 7 individuals each) only on the western breeding grounds, was also consistent with a missing component of the feeding grounds. The significant differentiation between Okinawa and Ogasawara, in both mtDNA and microsatellites, was also surprising given evidence of individual interchange based on photo-identification (Darling & Mori 1993, Barlow et al. 2011). Together, these results suggest that further investigation is required to describe population structure and to estimate abundance of what may be multiple breeding stocks in the western North Pacific

(Barlow et al. 2011). Like Central America and Mexico, the western North Pacific seems to have a complex migratory structure with some degree of mixing and some degree of isolation in different island regions. This substructure is particularly important given the known threats of unregulated exploitation along the coast of Japan and Korea (Baker et al. 2006, Lukoschek et al. 2009). For these reasons, and because of the relatively low estimates of abundance from SPLASH (Calambokidis et al. 2008), the International Union for the Conservation of Nature reports cause for concern for the western North Pacific stock (IUCN 2009).

Implications for management

As noted previously (Baker et al. 1998), the significant differentiation in mtDNA haplotypes among most feeding grounds and breeding grounds of the North Pacific indicates these are discrete populations, and satisfies Moritz's (Moritz 1994) criteria for recognition of 'management units', although not his criterion of reciprocal monophyly for recognition of 'evolutionarily significant units'. Additionally, we suggest the magnitude of differentiation in mtDNA haplotypes among breeding grounds, along with the significant differentiation in microsatellite alleles frequencies, is consistent with the criteria for 'significance' used in defining 'distinct population segments' (DPS) under the US Endangered Species Act (ESA; Waples 1991, 2006). Therefore, we believe that multiple lines of evidence support the recognition of 5 DPS related to breeding grounds of the North Pacific: Okinawa/ Philippines, a second West Pacific DPS (exact location unknown), Hawaii, Mexico and Central America (Fleming & Jackson 2011). In each case, these divisions are supported by significant differentiation in mtDNA haplotypes, as presented here and provided in advance for the global review humpback whales by Fleming & Jackson (2011), as well as microsatellite allele frequencies, as presented here. Mainland Mexico and the offshore Revillagigedo Islands can be considered discrete but not distinct given the low levels of genetic differentiation. Baja California appears to represent an area of mixing during migration by whales from both mainland and offshore breeding grounds of Mexico. The Ogasawara Islands of Japan are also likely to be an area of mixing for whales continuing on to Okinawa/ Philippines and to the Second West Pacific DPS. Recognition of a second West Pacific DPS, with unknown breeding grounds, would account for incon-

sistencies in mtDNA haplotype frequencies (see above) and rates of photo-identification resightings among the feeding grounds and breeding grounds of the western North Pacific (Calambokidis et al. 2008).

However, the potential to define genetically differentiated population segments on the feeding grounds, as well as the breeding grounds, differs from the tradition view of baleen whale stocks as reproductively isolated, migratory units defined by longitudinal boundaries (e.g. an Eastern, Central and Western stock of humpback whales; Donovan 1991). Although recognition of breeding stocks is most consistent with the criterion for reproductive isolation used in defining DPS under the ESA (Waples 2006), the observed assortment of mtDNA haplotypes on feeding grounds is strong evidence for recognizing unique maternal traditions of migration and habitat use as units to conserve (Taylor 2005). These maternal traditions are important components in the spatial recovery of whale populations in both seasonal habitats (Clapham et al. 2008) and in maintaining regional populations as functioning elements of their ecosystem.

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LITERATURE CITED

- Acebes JMV, Darling DJ, Yamaguchi M (2007) Status and distribution of humpback whales (*Megeptera novaeangliae*) in northern Luzon, Philippines. *J Cetacean Res Manag* 9:37–43
- Baker CS, Clapham P (2004) Modelling the past and future of whales and whaling. *Trends Ecol Evol* 19:365–371
- Baker CS, Medrano-Gonzalez L (2002) World-wide distribution and diversity of humpback whale mitochondrial DNA lineages. In: Pfeiffer CJ (ed) *Cell and molecular biology of marine mammals*. Krieger Publishing, Melbourne, FL, p 84–99
- Baker CS, Herman LM, Perry A, Lawton WS and others (1986) Migratory movement and population structure of humpback whales (*Megaptera novaeangliae*) in the central and eastern North Pacific. *Mar Ecol Prog Ser* 31: 105–119
- Baker CS, Perry A, Herman LM (1987) Reproductive histories of female humpback whales *Megaptera novaeangliae* in the North Pacific. *Mar Ecol Prog Ser* 41:103–114
- Baker CS, Palumbi SR, Lambertsen RH, Weinrich MT, Calambokidis J, O'Brien SJ (1990) Influence of seasonal migration on the distribution of mitochondrial DNA haplotypes in humpback whales. *Nature* 344:238–240
- Baker CS, Perry A, Bannister JL, Weinrich MT and others (1993) Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proc Natl Acad Sci USA* 90:8239–8243
- Baker CS, Slade RW, Bannister JL, Abernethy RB and others (1994) Hierarchical structure of mitochondrial DNA gene flow among humpback whales *Megaptera novaeangliae*, world-wide. *Mol Ecol* 3:313–327
- Baker CS, Medrano-Gonzalez L, Calambokidis J, Perry A and others (1998) Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. *Mol Ecol* 7:695–707
- Baker CS, Lukoschek V, Lavery S, Dalebout ML, Yong-un M, Endo T, Funahashi N (2006) Incomplete reporting of whale, dolphin and porpoise 'bycatch' revealed by molecular monitoring of Korean markets. *Anim Conserv* 9: 474–482
- Barlow J, Calambokidis J, Falcone EA, Baker CS and others (2011) Humpback whale abundance in the North Pacific estimated by photographic capture-recapture with bias correction from simulation studies. *Mar Mamm Sci* 27: 793–818
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 57:289–300
- Bérubé M, Jørgensen H, McEwing R, Palsbøll PJ (2000) Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, *Megaptera novaeangliae*. *Mol Ecol* 9:2181–2183
- Birky CW Jr, Maruyama T, Fuers P (1983) An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts and some results. *Genetics* 103:513–527
- Bolker BM, Okuyama T, Bjørndal KA, Bolten AB (2007) Incorporating multiple mixed stocks in mixed stock analysis: 'many-to-many' analyses. *Mol Ecol* 16:685–695
- Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. *Mol Ecol* 16:4886–4907
- Brown MR, Corkeron PJ, Hale PT, Schultz KW, Bryden MM (1995) Evidence for a sex segregated migration in the humpback whale (*Megaptera novaeangliae*). *Proc Biol Sci* 259:229–234
- Calambokidis J, Steiger GH, Evenson JR, Flynn KR and others (1996) Interchange and isolation of humpback whales off California and other feeding grounds in the North Pacific. *Mar Mamm Sci* 12:215–226
- Calambokidis J, Steiger GH, Straley JM, Herman LM and others (2001) Movements and population structure of humpback whales in the North Pacific Basin. *Mar Mamm Sci* 17:769–794
- Calambokidis J, Falcone EA, Quinn TJ, Burdin AM and others (2008) SPLASH: Structure of populations, levels of abundance and status of humpback whales in the North Pacific. US Department of Commerce, Western Administrative Center, Seattle, WA
- Carlsson J (2008) Effects of microsatellite null alleles on assignment testing. *J Hered* 99:616–623
- Carrroll E, Patenaude N, Alexander A, Steel D and others (2011) Population structure and individual movement of southern right whales around New Zealand and Australia. *Mar Ecol Prog Ser* 432:257–268

- Cerchio S, Jacobsen JK, Norris TF (2001) Temporal geographic variation in songs of humpback whales (*Megaptera novaeangliae*): synchronous change in Hawaiian and Mexican breeding assemblages. *Anim Behav* 62:313–329
- Clapham PJ, Aguilar A, Hatch LT (2008) Determining spatial and temporal scales for management: lessons from whaling. *Mar Mamm Sci* 24:183–201
- Darling JD, Cerchio S (1993) Movement of a humpback whale (*Megaptera novaeangliae*) between Japan and Hawaii. *Mar Mamm Sci* 9:84–88
- Darling JD, Jurasz CM (1983) Migratory destinations of North Pacific humpback whales (*Megaptera novaeangliae*). In: Payne R (ed) *Communication and behavior of whales*. Westview Press, Boulder, CO, p 359–368
- Darling JD, McSweeney DJ (1985) Observations on the migrations of North Pacific humpback whales (*Megaptera novaeangliae*). *Can J Zool* 63:308–314
- Darling JD, Mori K (1993) Recent observations of humpback whales (*Megaptera novaeangliae*) in Japanese waters off Ogasawara and Okinawa. *Can J Zool* 71:325–333
- Darling JD, Calambokidis J, Balcomb KC, Bloedel P and others (1996) Movement of a humpback whale (*Megaptera novaeangliae*) from Japan to British Columbia and return. *Mar Mamm Sci* 12:281–287
- Dobson FS (1982) Competition for mates and predominant juvenile male dispersal in mammals. *Anim Behav* 30:1183–1192
- Donovan GP (1991) A review of IWC stock boundaries. Report of the International Whaling Commission (Spec Issue) 13:39–68
- Ennos RA (1994) Estimating the relative rates of pollen and seed migration among plant populations. *Heredity* 72:250–259
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. *Mol Ecol* 14:2611–2620
- Ewing B, Green P (1998) Base-calling of automated sequencer traces using *Phred*. II. Error probabilities. *Genome Res* 8:186–194
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10:564–567
- Fleming A, Jackson JA (2011) Global review of humpback whales (*Megaptera novaeangliae*). NOAA Technical Memorandum NMFS-SWFSC-474
- Garrigue C, Dodemont R, Steel D, Baker CS (2004) Organismal and 'gametic' capture-recapture using microsatellite genotyping confirm low abundance and reproductive autonomy of humpback whales on the wintering grounds of New Caledonia. *Mar Ecol Prog Ser* 274:251–262
- Gilson A, Syvanen M, Levine K, Banks J (1998) Deer gender determination by polymerase chain reaction: validation study and application to tissues, bloodstains, and hair forensic samples from California. *Calif Fish Game* 84:159–169
- Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Mol Ecol* 11:1103–1114
- Greenwood PJ (1980) Mating systems, philopatry, and dispersal in birds and mammals. *Anim Behav* 28:1140–1162
- Greenwood PJ (1983) Mating systems and the evolutionary consequences of dispersal. In: Swingland IR, Greenwood PJ (eds) *The ecology of animal movement*. Clarendon Press, Oxford, p 116–131
- Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution* 59:1633–1638
- Hedrick PW, Allendorf FW, Baker CS (2013) Estimation of male gene flow from measures of nuclear and female genetic differentiation. *J Hered* 104:713–717
- Hess MA, Rhydderch JG, LeClair LL, Buckley RM, Kawase M, Hauser L (2012) Estimation of genotyping error rate from repeat genotyping, unintentional recaptures and known parent–offspring comparisons in 16 microsatellite loci for brown rockfish (*Sebastes auriculatus*). *Mol Ecol Resour* 12:1114–1123
- IUCN (2009) The IUCN Red List of Threatened Species. www.iucnredlist.org/details/13006/0
- Johnson JH, Wolman AA (1984) The humpback whale. *Mar Fish Rev* 46:30–37
- Jorgensen SJ, Reeb CA, Chapple TK, Anderson S and others (2010) Philopatry and migration of Pacific white sharks. *Proc R Soc B* 277:679–688
- Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* 16:1099–1106
- Lambertsen RH (1987) A biopsy system for large whales and its use for cytogenetics. *J Mammal* 68:443–445
- Latch E, Dharmarajan G, Glaubitz J, Rhodes O (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conserv Genet* 7:295–302
- Lohmann KJ, Putman NF, Lohmann CMF (2008) Geomagnetic imprinting: a unifying hypothesis of long-distance natal homing in salmon and sea turtles. *Proc Natl Acad Sci USA* 105:19096–19101
- Lukoschek V, Funahashi N, Lavery S, Dalebout ML, Cipriano F, Baker CS (2009) High proportion of protected minke whales sold on Japanese markets is due to illegal, unreported or unregulated exploitation. *Anim Conserv* 12:385–395
- Medrano-Gonzalez L, Aguayo-Lobo A, Urban-Ramirez J, Baker CS (1995) Diversity and distribution of mitochondrial DNA lineages among humpback whales, *Megaptera novaeangliae*, in the Mexican Pacific. *Can J Zool* 73:1735–1743
- Meirmans PG, Hedrick PW (2011) Assessing population structure: F_{ST} and related measures. *Mol Ecol Resour* 11:5–18
- Meirmans PG, Van Tienderen PH (2004) Genotype and Genodive: two programs for the analysis of genetic diversity of asexual organisms. *Mol Ecol Notes* 4:792–794
- Morin PA, Martien KK, Archer FI, Cipriano F, Steel D, Jackson J, Taylor BL (2010) Applied conservation genetics and the need for quality control and reporting of genetic data used in fisheries and wildlife management. *J Hered* 101:1–10
- Moritz C (1994) Defining 'Evolutionarily Significant Units' for conservation. *Trends Ecol Evol* 9:373–375
- Nakagawa S (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav Ecol* 15:1044–1045
- Narum S (2006) Beyond Bonferroni: Less conservative analyses for conservation genetics. *Conserv Genet* 7:783–787
- Olavarria C, Baker CS, Garrigue C, Poole M and others (2007) Population structure of humpback whales throughout the South Pacific and the origins of the eastern Polynesian breeding grounds. *Mar Ecol Prog Ser* 330:257–268

- Palsbøll PJ, Clapham PJ, Mattila DK, Larsen F and others (1995) Distribution of mtDNA haplotypes in North Atlantic humpback whales: the influence of behaviour on population structure. *Mar Ecol Prog Ser* 116:1–10
- Palsbøll PJ, Bérubé M, Larsen AH, Jørgensen H (1997) Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. *Mol Ecol* 6:893–895
- Palumbi SR, Baker CS (1994) Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. *Mol Biol Evol* 11:426–435
- Pardini AT, Jones CS, Noble LR, Kreiser B and others (2001) Sex-biased dispersal of great white sharks. *Nature* 412: 139–140
- Patenaude NJ, Portway VA, Schaeff CM, Bannister JL and others (2007) Mitochondrial DNA diversity and population structure among southern right whales (*Eubalaena australis*). *J Hered* 98:147–157
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6:288–295
- Pearce JM (2007) Philopatry: a return to origins. *Auk* 124: 1085–1087
- Pomilla C, Rosenbaum HC (2005) Against the current: an inter-oceanic whale migration event. *Biol Lett* 1:476–479
- Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes, consequences and solutions. *Nat Rev Genet* 6:847–859
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959
- Pusey AE (1987) Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends Ecol Evol* 2:295–299
- Quinn TP (2005) The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle, WA
- Robbins J, Dalla Rosa L, Allen JM, Mattila DK and others (2011) Return movement of a humpback whale between the Antarctic Peninsula and American Samoa: a seasonal migration record. *Endang Species Res* 13:117–121
- Rousset F (2008) Genepop, '007: a complete re-implementation of the Genepop software for Windows and Linux. *Mol Ecol Resour* 8:103–106
- Shields WM (1982) Philopatry, inbreeding and the evolution of sex. State University of New York Press, Albany, NY
- Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science* 236:787–792
- Steiger GH, Calambodikis J (1991) Movement of humpback whales between California and Costa Rica. *Mar Mamm Sci* 7:306–310
- Stevick PT, Neves MC, Johansen F, Engel MH, Allen J, Marcondes MCC, Carlson C (2011) A quarter of a world away: female humpback whale moves 10 000 km between breeding areas. *Biol Lett* 7:299–302
- Taylor BL (2005) Identifying units to conserve. In: Reynolds JE III, Perrin WF, Reeves RR, Montgomery S, Ragen TJ (eds) *Marine mammal research: conservation beyond crisis*. The Johns Hopkins University Press, Baltimore, MD, p 149–164
- Taylor BL, Chivers SJ (1997) Anonymous alleles can bias results. In: Dizon AE, Chivers SJ, Perrin WF (eds) *Molecular genetics of marine mammals: special issue of the Society of Marine Mammology*. Allen Press, La Jolla, CA, p 341–346
- Taylor BL, Dizon AE (1996) The need to estimate power to link genetics and demography for conservation. *Conserv Biol* 10:661–664
- Taylor BL, Chivers SJ, Dizon AE (1997) Using statistical power to interpret genetic data to define management units for marine mammals. In: Dizon AE, Chivers SJ, Perrin WF (eds) *Molecular genetics of marine mammals: special issue of the Society of Marine Mammology*. Allen Press, La Jolla, CA, p 347–364
- Taylor BL, Chivers SJ, Laresse J, Perrin WF (2007) Generation length and percent mature estimates for IUCN assessments of cetaceans. Administrative Report LJ-07-01, National Marine Fisheries Service, Southwest Fisheries Science Center, La Jolla, CA
- Urbán J, Jaramillo A, Aguayo A, Guevara PL and others (2000) Migratory destinations of humpback whales wintering in the Mexican Pacific. *J Cetacean Res Manag* 2: 101–110
- Valsecchi E, Amos W (1996) Microsatellite markers for the study of cetacean populations. *Mol Ecol* 5:151–156
- van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538
- Waits JL, Leberg PL (2000) Biases associated with population estimation using molecular tagging. *Anim Conserv* 3:191–199
- Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol Ecol* 10:249–256
- Waldick RC, Brown MW, White BN (1999) Characterization and isolation of microsatellite loci from the endangered North Atlantic right whale. *Mol Ecol* 8:1763–1765
- Waples RS (1991) Pacific salmon, *Oncorhynchus* spp., and the definition of 'species' under the Endangered Species Act. *Mar Fish Rev* 53:11–22
- Waples RS (2006) Distinct population segments. In: Scott JM, Goble DD, Davis FW (eds) *The Endangered Species Act at thirty: conserving biodiversity in human-dominated landscapes*, Vol 2. Island Press, Washington, DC, p 127–149
- Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Mol Ecol* 15:1419–1439
- Whitehead H, Rendell L, Osborne RW, Würsig B (2004) Culture and conservation of non-humans with reference to whales and dolphins: review and new directions. *Biol Conserv* 120:427–437
- Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. *Heredity* 82: 117–125
- Witteveen BH, Straley JM, von Ziegesar O, Steel D, Baker CS (2004) Abundance and mtDNA differentiation of humpback whales (*Megaptera novaeangliae*) in the Shumagin Islands, Alaska. *Can J Zool* 82:1352–1359
- Wright S (1951) The genetical structure of populations. *Ann Eugen (= Ann Hum Gen)* 15:323–354