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What influences the worldwide genetic structure of sperm whales (*Physeter macrocephalus*)?

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Abstract

The interplay of natural selection and genetic drift, influenced by geographic isolation, mating systems and population size, determines patterns of genetic diversity within species. The sperm whale provides an interesting example of a long-lived species with few geographic barriers to dispersal. Worldwide mtDNA diversity is relatively low, but highly structured among geographic regions and social groups, attributed to female philopatry. However, it is unclear whether this female philopatry is due to geographic regions or social groups, or how this might vary on a worldwide scale. To answer these questions, we combined mtDNA information for 1091 previously published samples with 542 newly obtained DNA profiles (394-bp mtDNA, sex, 13 microsatellites) including the previously unsampled Indian Ocean, and social group information for 541 individuals. We found low mtDNA diversity (π = 0.430%) reflecting an expansion event <80 000 years bp, but strong differentiation by ocean, among regions within some oceans, and among social groups. In comparison, microsatellite differentiation was low at all levels, presumably due to male-mediated gene flow. A hierarchical AMOVA showed that regions were important for explaining mtDNA variance in the Indian Ocean, but not Pacific, with social group sampling in the Atlantic too limited to include in analyses. Social groups were important in partitioning mtDNA and microsatellite variance within both oceans. Therefore, both geographic philopatry and social philopatry influence genetic structure in the sperm whale, but their relative importance differs by sex and ocean, reflecting breeding behaviour, geographic features and perhaps a more recent origin of sperm whales in the Pacific. By investigating the interplay of evolutionary forces operating at different temporal and geographic scales, we show that sperm whales are perhaps a unique example of a worldwide population expansion followed by rapid assortment due to female social organization.

Keywords: Cetacea, microsatellite genotypes, mtDNA, population expansion, population genetics, sex-biased dispersal

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Introduction

Despite the absence of obvious geographic barriers, striking patterns of genetic differentiation and diversity

are evident in many marine megafauna. This includes low genetic diversity due to past population bottlenecks/expansions (e.g. giant squid, Winkelmann *et al.* 2013; killer whales, Moura *et al.* 2014); strong patterns of genetic differentiation due to prey specialization (e.g. killer whales, Riesch *et al.* 2012) or habitat specialization (e.g. sea lions, Lowther *et al.* 2012; harbour porpoises, Fontaine *et al.* 2014); genetic differentiation due to maternal or natal fidelity to breeding locations and migration routes (e.g. turtles, Bowen *et al.* 1992; baleen whales, Baker *et al.* 2013); and male-biased gene flow, as reflected in biparentally inherited nuclear markers and maternally inherited mitochondrial DNA (e.g. great white sharks, Pardini *et al.* 2001; humpback whales, Baker *et al.* 2013). In some species (e.g. killer whales, sperm whales), social groups also influence genetic differentiation, potentially reinforced by culture such as vocal dialects (Hoelzel *et al.* 2007; Whitehead *et al.* 2012; Cantor & Whitehead 2015; Cantor *et al.* 2015; Gero *et al.* 2015). While studies often investigate single factors that influence genetic diversity, teasing apart different mechanisms requires an assessment of genetic diversity patterns over multiple spatial and temporal time scales.

Due to its worldwide distribution (Gosho *et al.* 1984), social behaviour (Gero *et al.* 2015) and acoustically mediated culture (Cantor & Whitehead 2015; Cantor *et al.* 2015), the sperm whale presents an interesting case study for this type of hierarchical analysis. Although whaling removed hundreds of thousands of individuals (Best 1979; Whitehead 2002, 2003), the sperm whale is relatively abundant in comparison with other large whale species (~360 000 individuals worldwide; Whitehead 1998, 2002). Given the sperm whale's abundance and wide geographic range, mitochondrial DNA (mtDNA) diversity in sperm whales is relatively low compared with many other cetacean species (Lyrholm *et al.* 1996; Whitehead 1998; Alexander *et al.* 2013), yet marked by moderate-to-strong differentiation between oceans (Lyrholm & Gyllensten 1998), among marginal seas within the Atlantic (Drouot *et al.* 2004; Engelhaupt *et al.* 2009) and among social groups within the Pacific (Lyrholm & Gyllensten 1998; Rendell *et al.* 2012). In an analysis of mitogenomes (Alexander *et al.* 2013), three previously proposed hypotheses were considered as the most likely causes of the low mtDNA diversity in sperm whales: a population bottleneck and/or expansion (Lyrholm *et al.* 1996; Lyrholm & Gyllensten 1998); a selective sweep due to a favourable substitution in a mtDNA-encoded protein (Janik 2001); or a selective sweep due to beneficial cultural traits transmitted matrilineally in parallel with the mitogenome – cultural hitchhiking (Whitehead 1998, 2005). In comparison, although significant nuclear differentiation (based on microsatellite genotypes) has been observed among social groups in the Pacific (Lyrholm *et al.* 1999), there is only weak differentiation among regions within oceans (Engelhaupt *et al.* 2009; Mesnick *et al.* 2011), and no significant nuclear differentiation between ocean basins (Lyrholm *et al.* 1999). The contrast between mtDNA and microsatellite differentiation has been interpreted as male dispersal and female philopatry at three hierarchical levels: oceanic scales (Lyrholm *et al.* 1999), between regions within oceans (e.g. the Atlantic; Engelhaupt *et al.* 2009) and at the social group level (Lyrholm & Gyllensten 1998).

Female philopatry and male-biased dispersal are consistent with behavioural observations of sperm whale social structure. Males disperse from their natal social units at an age of 3–15 years (Best 1979; Richard *et al.* 1996a; Whitehead 2003) and become increasingly solitary as they age, extending their latitudinal range into polar waters (Best 1979; Allen 1980; Whitehead 2003). After reaching social maturity (at 25–27 years, Best 1979), males associate with females for the purposes of mating, but do not permanently remain with any given female social group (Whitehead 1993, 1994; Richard *et al.* 1996a). Female social groups contain adult females that show long-term social bonds with one another, as well as juveniles of both sexes, and are confined to low-latitude tropical and temperate waters (Best 1979; Richard *et al.* 1996a; Christal *et al.* 1998; Dufault & Whitehead 1998; Dufault *et al.* 1999; Coakes & Whitehead 2004). However, there are substantial differences by ocean in vocal dialects, female social group size and proportion of calves within social groups (Whitehead *et al.* 2012; Gero *et al.* 2015), suggesting that the relative importance of female social groups in partitioning genetic diversity might vary by ocean.

Here, we investigate the cause of the rapid radiation of maternal lineages in sperm whales, and how social group and geographic factors partition genetic diversity in different oceans. We hypothesize that the low but highly structured mtDNA diversity observed in the sperm whale is consistent with a recent, rapid radiation of a single mtDNA lineage, followed by genetic drift due to female philopatry at regional and social group levels. For this, we assembled the largest sperm whale genetic data set to date, including both published and previously unpublished data. Previously unpublished data included stranding samples, and samples collected by the 'Voyage of the *Odyssey*': a 5-year expedition that collected biopsy samples (skin and blubber) from undersampled equatorial regions (Fig. 1), including the previously unsampled Indian Ocean (Godard *et al.* 2003). Previously published data included mtDNA control region (CR) haplotypes from 1,091 samples (Richard *et al.* 1996a; Lyrholm & Gyllensten 1998;

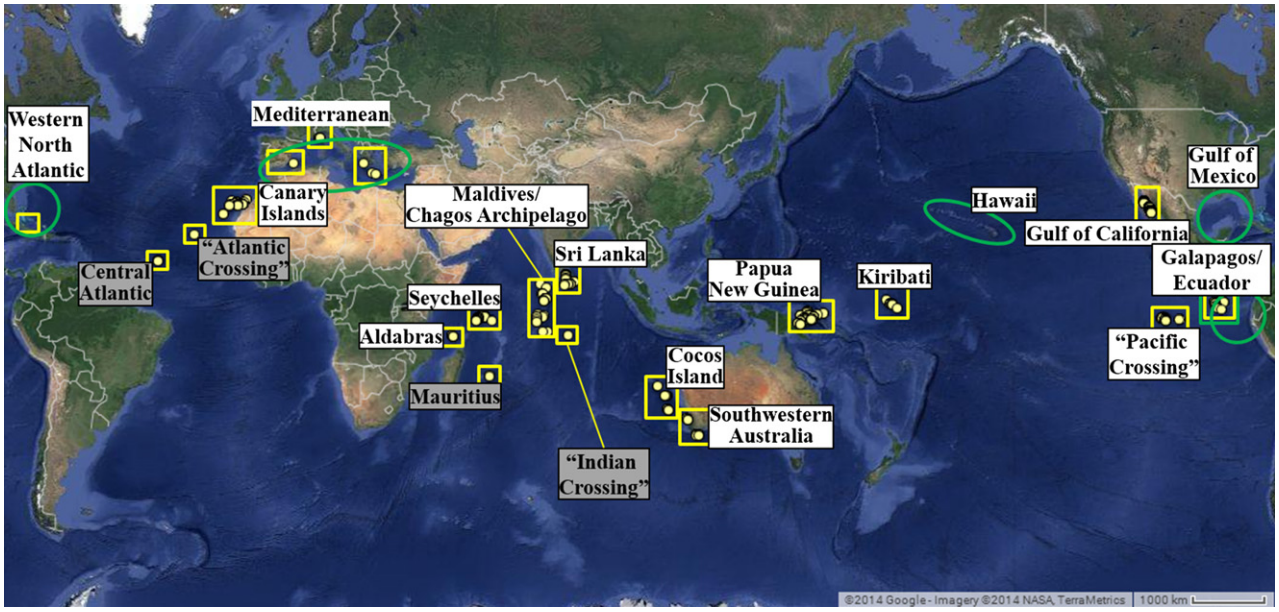


Fig. 1 Distribution of genetic samples from sperm whales used in regional analyses. White labels indicate areas included in within-ocean mtDNA and microsatellite tropical/subtropical regional analyses. *Odyssey* samples were aggregated together if they occurred within 500 km of another sample. This created the localized regional areas shown in the rectangles. Additional mtDNA samples/regions included in analyses originating from previous studies collected over similar spatial scales are circled (references in Table 1). Grey labels show regions not included in regional analyses due to small sample sizes.

Whitehead *et al.* 1998; Engelhaupt *et al.* 2009; Mesnick *et al.* 2011; Rendell *et al.* 2012). Using this unprecedented data set, we first explicitly test the hypothesis that a past population expansion could explain the low mtDNA diversity observed in the sperm whale. We then evaluate the importance of geographic regions vs. social groups in determining genetic structure within the Pacific and Indian Oceans (where sufficient individuals with social group information were available), using the unique circumequatorial collection of samples from social groups and within-ocean regions. Finally, we also examine sex-specific differences by carrying out these analyses for both biparentally inherited nuclear markers (13 microsatellite loci) and the maternally inherited mtDNA. This study demonstrates how different factors shape patterns of genetic diversity at multiple scales in a broadly distributed marine organism.

Materials and methods

Assembly of mtDNA data set and definition of spatial scales

Using the definitions developed by Mesnick *et al.* (2005, also see Appendix S1, Supporting information), we summarized mtDNA haplotype information from previous publications by ocean and within-ocean region (Appendix S2, Supporting information). Regions were defined by aggregating samples that were obtained within ~500 km of each other, with the exception of the Mediterranean that was pooled over the entire sea for consistency with previous publications (Engelhaupt *et al.* 2009). Areas included in regional analyses were restricted to those sampled by the *Odyssey* (and augmented by samples from previous studies, where available), and tropical/subtropical areas (38°S to 38°N) sampled in previous studies, as these were the latitudes primarily sampled by the *Odyssey*. Regions were also required to have five or more sampled individuals to limit the effect of low sample sizes. Aggregation of data sets from different publications was possible because of the concerted efforts of the Cachalote Consortium (Mesnick *et al.* 2005) to standardize nomenclature for sperm whale mtDNA CR haplotypes. A lack of standardized nuclear markers did not allow for the identification and removal of potential between-study replicates. However, we removed within-study replicates where identified.

'Voyage of the *Odyssey*' samples were collected from 1999 to 2005 in circum-equatorial regions (Fig. 1) using a biopsy dart. As detailed in Godard *et al.* (2003), total genomic DNA was extracted from the *Odyssey* samples using a high-salt procedure. DNA aliquots of 895 samples were then provided by Ocean Alliance, sponsor of the 'Voyage of the *Odyssey*'. New Zealand sperm whale skin and tissue samples (n = 89) were collected from strandings by New Zealand Department of Conservation staff from 1994 to 2008 and archived in the New Zealand Cetacean Tissue Archive (CeTA) at the University of

Auckland. One sample originating in Samoa, archived in CeTA, was also included. Oregon sperm whale skin and tissue samples (n = 3) were provided by the Oregon Marine Mammal Stranding Network. DNA was extracted from samples of stranded animals following a standard phenol/chloroform technique (Sambrook *et al.* 1989) as modified by Baker *et al.* (1994).

We carried out amplification of the mtDNA CR using the primers M13dlp1.5 and tphe and sequenced a 619-bp consensus length of this fragment on an ABI3730xl DNA analyser, as described in Alexander *et al.* (2013). Sequences were trimmed using PHRED scores and by eye in SEQUENCHER v. 4.6 (Gene Codes). After trimming, sequences with more than 10% of bases showing a PHRED score of <20 were resequenced or removed from the data set (Morin *et al.* 2010). We visually confirmed variable sites between haplotypes in each sequence using SEQUENCHER. After removal of replicates, we trimmed the *Odyssey* and stranding samples to the shorter consensus length of 394 bp and combined them with the previously published mtDNA data. This 394-bp fragment has the highest level of diversity across the sperm whale mitogenome and accurately reflects intraspecific phylogenies based on the full mitogenome sequence (Alexander *et al.* 2013).

Sex identification of Odyssey and stranding samples

We sexed samples using a multiplexed PCR amplifying 152 bp of the *SRY* on the Y chromosome of males (Richard *et al.* 1994; primers: sperm whale-specific *SRY* primers), and a 442- to 445-bp fragment of the *ZFX/ZFY* fragment present in both males and females (Aasen & Medrano 1990; primers: P1-5EZ and P2-3EZ). Each reaction consisted of 1 μL of sample DNA, and a final concentration of 0.9× Platinum *Taq* buffer (Invitrogen), 0.36 μM of each of the four primers, 2.27 mM $MgCl_2$, 0.18 mM dNTP and 0.25 U of Platinum *Taq* polymerase (Invitrogen), with ddH_2O to 11 μL total volume. The temperature profile consisted of an initial denaturing step of 3 min at 94 °C, followed by 35 cycles of 94 °C for 45 s, 58 °C for 45 s and 72 °C for 60 s, followed by a final extension step of 72 °C for 10 min. The PCR products were run on a 1.6% agarose gel (buffer: TBE), stained with ethidium bromide and visualized under UV light. The presence of two bands indicated a male sample, one band a female sample and no bands PCR failure.

Microsatellite genotyping, identification of replicates and kin

Thirteen microsatellite loci were selected based on previous genotyping in the sperm whale (Engelhaupt *et al.* 2009) and other cetacean species (Appendix S3, Supporting information). We amplified each locus in an individual reaction, with 1 μL of the sample DNA, a final concentration of 0.9× Platinum *Taq* buffer (Invitrogen), 0.36 μM of each primer and 0.18 mM dNTP. $MgCl_2$ and Platinum *Taq* polymerase (Invitrogen) concentrations varied by locus as detailed in Appendix S3 (Supporting information), and we added ddH_2O to 11 μL total volume. Temperature profiles consisted of an initial denaturing step of 3–5 min at 94–95 °C, followed by 35–40 cycles of 94–95 °C for 30–40 s, the locus-specific annealing temperature (as detailed in Appendix S3, Supporting information) for 30–60 s, and 72 °C for 30–60 s, followed by a final extension step of 72 °C for 8–30 min. Multiple microsatellite loci were combined based on differing size range and fluorescent label (Appendix S3, Supporting information) and coloaded on an ABI3730xl DNA Analyzer with GS500 LIZ ladder. Output was processed using GENEMAPPER v. 3.7 (Applied Biosystems), with a minimum signal strength detection threshold of 50 units. All automated calls were checked by eye, with a subset of samples cross-checked by a second researcher (D. Steel) to ensure consistency in allele calling.

Other quality control (QC) measures were then carried out following Morin *et al.* (2010). Samples were only included if they were genotyped for at least eight microsatellite loci and successfully sexed or sequenced for mtDNA CR. This QC was to limit the inclusion of samples likely affected by low DNA quantity/quality. Identification of replicate samples was carried out using CERVUS v. 3.0 (Kalinowski *et al.* 2007). To compensate for genotyping error, we used relaxed matching allowing for mismatches at up to four microsatellite loci, with mismatching loci corrected or repeated. If remaining mismatches were consistent with allelic dropout, samples were considered replicates if they matched at sex and mtDNA CR. Probability of identity ($p_{(ID)}$) was calculated using CERVUS for pairs showing exact matches, and GENALEX v. 6.501 (Peakall & Smouse 2006, 2012) for those with mismatches (average $p_{(ID)}$ for the combination of exactly matching markers). The per-allele microsatellite error rate (Pompanon *et al.* 2005) was estimated using intentional duplicates for 110 samples, selected randomly with respect to DNA quality and quantity. Tests for deviation from Hardy–Weinberg equilibrium and for linkage disequilibrium were conducted using GENEPOP v. 4.2 (Raymond & Rousset 1995a), and tests for the presence of large-allele dropout and null alleles using MICRO-CHECKER v. 2.2.3 (van Oosterhout *et al.* 2004), following the methods of Carroll *et al.* (2011). We excluded microsatellite loci from population genetic analyses if they showed departures from Hardy–Weinberg equilibrium. A 'kin-restricted' data set was created to account for the sampling of first-order

relatives within social groups. We followed Mesnick's *et al.* (2011) approach of removing one member of every first-order kin pair (defined as samples sharing at least one allele at every microsatellite locus) identified using SOLOMON v. 1.0-1 (Christie *et al.* 2013). We retained the sample with the most complete genotype from each pair. We conducted analyses on both the 'full' and 'restricted' versions of this data set.

Testing for a population expansion

We tested for a population expansion in the mtDNA data set by assessing Fu's *F*s (Fu 1997) and the mismatch distribution (Slatkin & Hudson 1991; Rogers & Harpending 1992; Harpending 1994) under a demographic expansion scenario through ARLEQUIN v. 3.5 with 10 000 permutations to assess significance (Schneider & Excoffier 1999; Excoffier *et al.* 2005). We carried out these tests at the worldwide, oceanic and regional levels.

mtDNA diversity, differentiation and phylogeography

Haplotype and nucleotide diversity (using the Tamura & Nei (1993) correction) were calculated using ARLEQUIN. Differences in haplotype diversity and nucleotide diversity between oceans were assessed using a custom R v. 3.0.2 (R Core Team 2013) script, to conduct a permutation test with 10 000 replicates (Alexander 2015). We inferred the number of substitutions between the mtDNA CR haplotypes with a parsimony network created using TCS v. 1.2.1 (Clement *et al.* 2000). We examined oceanic differentiation using F_{ST} and Φ_{ST} (with the Tamura & Nei (1993) correction), with 10 000 replicates to assess significance in ARLEQUIN. To evaluate the potential influence of phylogeographic structure (i.e. divergence as well as drift), we used PERMUT v. 2.0 (Petit 2010) to test for differences between G_{ST} and N_{ST} (analogs of F_{ST} and Φ_{ST}: Pons & Petit 1996).

Microsatellite diversity and differentiation

Observed heterozygosity and expected heterozygosity were calculated using CERVUS and allelic richness using FSTAT v. 2.9.3 (Goudet 2001). We tested for significant differences in observed heterozygosity and allelic richness between oceans using a custom R script (Hamner 2014) that implemented *t*-tests or Wilcoxon signed rank tests depending on equality of variances and normality of differences between areas (Appendix S4, Supporting information). We calculated F_{IS} by region and ocean (using oceanic and worldwide microsatellite allele frequencies, respectively) using FSTAT, with 10 000 replicates to assess significance. To examine oceanic differentiation, we calculated F_{ST} through GENEPOP (Raymond & Rousset 1995b; Rousset 2008), using the exact test to assess significance (Raymond & Rousset 1995a). G''_{ST}, an index that compensates for the diversity of microsatellites (Meirmans & Hedrick 2011), was calculated with GENODIVE v. 2.ob25 (Meirmans & van Tienderen 2004), using 10 000 permutations to assess significance. The presence of population structure independent of our a priori partitions was assessed for the regional microsatellite data set using STRUCTURE v 2.3.4 (Pritchard *et al.* 2000; Falush *et al.* 2003). Following Engelhaupt *et al.* (2009), we assumed admixture and correlated allele frequencies with 500 000 burn-in steps, followed by 1 000 000 steps. Twenty replicates (following the recommendations of Gilbert *et al.* 2012) were carried out for $K = 1$ to $K = 13$. The Evanno *et al.* (2005) method was used to assess the best fitting K through STRUCTURE HARVESTER (Earl & vonHoldt 2012). Using STRUCTURE HARVESTER output, CLUMPP (Jakobsson & Rosenberg 2007) was used to align cluster assignment across replicates.

Evaluating the relative importance of social group vs. geographic regions by ocean

We used field data on spatial and temporal proximity of *Odyssey* biopsy samples to identify samples collected during a single encounter with a social group. To account for previously sampled groups that were unintentionally re-encountered, we combined groups that had genetic replicates between them. Our groups likely correspond to a mix of 'social groups' and 'social units' as defined in previous publications (Christal *et al.* 1998; Whitehead 2003; Gero *et al.* 2015). We included social groups from the literature where mtDNA data were available (Appendix S5, Supporting information). Tests of genetic differentiation were conducted partitioning the data set by social group, for groups where two or more individuals passed QC. Hierarchical analyses nesting social group within ocean and region at the worldwide scale, and within region for the Pacific and Indian Oceans (where adequate numbers of groups were available), were conducted through ARLEQUIN for mtDNA (F_{ST} and Φ_{ST}) and GDA v 1.0 (Lewis & Zaykin 2001) for microsatellites (F_{ST} only). To limit the effect of small sample sizes, nested analyses were restricted to a subset of social groups that had five or more individuals pass QC.

Testing for female philopatry: sex-biased gene flow and dispersal

We restricted analyses of sex-biased gene flow and dispersal to samples genotyped in this study, where sex information was available. Analyses were carried out at

the oceanic and within-ocean regional levels (including all oceans/regions with at least 2 individuals of each sex) following the methods of Oremus *et al.* (2007). Due to limited numbers of social groups with two or more sampled males (Appendix S5, Supporting information), no analyses were conducted at the social group level. We investigated sex-biased dispersal using two methods in FSTAT: (1) a comparison of sex-specific F_{ST} values for both mtDNA (coding the mtDNA CR as a homozygote locus) and microsatellites and (2) calculation of the sex-specific variance of assignment index (vAIc) based on microsatellites (Goudet *et al.* 2002). We tested the difference between sex-specific values using 10 000 permutations. The more dispersive sex is expected to have a lower F_{ST} value (method 1), but higher variance (method 2), than the more philopatric sex (Oremus *et al.* 2007). We note that males in this data set included immature males that had not dispersed from their natal social group that could conservatively bias the tests against finding male-biased dispersal. As well as sex-biased dispersal, we obtained sex-specific gene flow estimates using the formulas presented in Hedrick *et al.* (2013) and microsatellite/mtDNA CR F_{ST} as the input values.

Results

Assembly of mtDNA data set

We summarized sequence information for 1091 samples from previous studies (Richard *et al.* 1996a; Lyrholm & Gyllensten 1998; Whitehead *et al.* 1998; Engelhaupt *et al.* 2009; Mesnick *et al.* 2011; Rendell *et al.* 2012; as detailed in Appendix S2, Supporting information). After the removal of replicates identified by genotyping, mtDNA sequences were available for 496 individuals in the collection from the *Odyssey* and from strandings. These sequences were trimmed to a consensus sequence length of 394 bp and combined with the previously published information resulting in 1587 sequences included in analyses of mtDNA differentiation and diversity at the worldwide and oceanic level. Of these, 998 samples were included in analyses of 16 regions within oceans (Table 1).

Assembly of microsatellite data set and quality control

Of the 988 total samples genotyped in this study, 671 passed quality control, with a minimum of 8 microsatellite loci each. On average, the samples passing QC had microsatellite genotypes that were 92.8% complete (SD = 9.58%), representing 12 out of a potentially complete genotype of 13 microsatellite loci. We identified replicates using between 6 and 13 overlapping loci with $p_{(ID)}$s between 3.39E-21 and 1.76E-06, and $p_{(ID\text{-}sibs)}$ between 2.16E-06 and 4.50E-03. The per-allele microsatellite error rate was 1.27% based on 74 intentional duplicate pairs that passed QC. This error was largely due to allelic dropout (>95%) that was then identified and corrected, and was similar in magnitude to previous studies on sperm whales and other cetaceans (Carroll *et al.* 2011; Mesnick *et al.* 2011; Baker *et al.* 2013). Using the known duplicates, there was no detectable error in designation of mtDNA haplotypes (i.e. an error rate of <0.7%) and only one male/female discrepancy between a duplicate pair (e.g. an error rate of 1.69%).

After the removal of replicates, the 671 genotypes that passed QC represented 542 individuals. Using SOLOMON to identify pairs of individuals sharing an allele at every locus, we found 12 likely first-order kin relationships. One pair from the Chagos Archipelago consisted of two males, with the remaining relationships involving at least one female. Given the small number of identified potential first-order kin, results for the 'full' and 'kin-restricted' data sets were very similar for all analyses. Consequently, we provide results of the 'restricted' data set only as Appendices S6 and S7 (Supporting information). We defined 13 within-ocean regions in the microsatellite data set (Table 1). In contrast with the mtDNA data set, three regions were not represented as they were not genotyped in this current study/had insufficient numbers of individuals pass QC: Hawai'i, western North Atlantic, and the Gulf of Mexico. We did not detect consistent significant deviations from Hardy–Weinberg equilibrium or linkage disequilibrium across the within-ocean regions. In addition, we found no consistent evidence of scoring error, preferential large-allele dropout, or null alleles for any microsatellite loci across the within-ocean regions. Therefore, we retained all loci (Table 2) for analyses of microsatellite diversity and differentiation.

Testing for a population expansion

For the worldwide data set, Fu's *F*s was strongly and significantly negative (*F*s = −25.4, $P = 0.0002$), and the mtDNA mismatch distribution appeared unimodal (Fig. 2; parameter estimates and *P*-values for all comparisons listed in Appendix S8, Supporting information). Along with a star-like mtDNA network (Fig. 3), these results are strong indicators of a worldwide population expansion in female lineages of the sperm whale (Slatkin & Hudson 1991). Based on $\tau = 1.625$ and a control region substitution rate of 2.6%/million years (Alexander *et al.* 2013), the age of the expansion event was estimated at 78 300 years ago (95% CI: 72 300–97 900), although the SSD and raggedness indices suggest the expansion model simulated by ARLEQUIN was not a good fit for the data ($P < 0.0109$).

Table 1 Regional, oceanic and worldwide sample sizes (*n*) and diversity metrics for mtDNA and microsatellites. For mtDNA, number of haplotypes (*k*), haplotype diversity (*h*) and nucleotide diversity (in %, π) are presented, with standard deviations calculated in ARLEQUIN. For microsatellites, numbers of individuals are given by sex (*F*, *M*) and total sample size (*n*)

		mtDNA				Microsatellites						
	Geographic area	*n*	*k*	*h* (SD)	π (SD)	*F*	*M*	*n*	Allelic richness	H_O	F_{IS}	References
Pacific	Gulf of California	122	11	0.788 (0.024)	0.368 (0.250)	93**	20	122	5.2	0.702	0.016	[1]
	Galapagos/Ecuador	285	16	0.744 (0.012)	0.352 (0.240)	0	23**	23	5.1	0.677	0.031	[1, 2, 3, 4, 5]
	Pacific Crossing	36	8	0.679 (0.071)	0.301 (0.220)	20	13	37	5.1	0.704	0.013	[1]
	Hawai'i	28	4	0.643 (0.068)	0.195 (0.164)	—	—	—	—	—	—	[6]
	Kiribati	13	4	0.718 (0.089)	0.381 (0.276)	10*	2	13	5.3	0.684	0.092*	[1]
	Papua New Guinea	63	8	0.720 (0.036)	0.299 (0.216)	54**	8	65	5.1	0.687	0.031	[1]
	Unassigned Pacific	478	—	—	—	22	31	66	—	—	—	[1, 2, 3, 5, 6]
	Total	1025	33	0.780 (0.008)	0.385 (0.256)	199**	97	326	8.9	0.704	0.021*	—
Indian	Southwestern Australia	21	5	0.791 (0.044)	0.305 (0.226)	9	4	21	5.1	0.697	0.007	[1]
	Cocos Island	18	3	0.451 (0.117)	0.229 (0.187)	18**	0	18	5.2	0.712	0.001	[1]
	Sri Lanka	42	3	0.382 (0.076)	0.131 (0.125)	42**	6	56	5.1	0.671	0.040*	[1]
	Maldives/Chagos Archipelago	33	4	0.570 (0.061)	0.300 (0.220)	9	15	34	5.3	0.700	0.041	[1]
	Seychelles	31	6	0.716 (0.066)	0.407 (0.276)	17**	2	31	5.3	0.697	0.020	[1]
	Aldabras	12	3	0.712 (0.069)	0.362 (0.267)	6	2	12	5.1	0.677	0.027	[1]
	Unassigned Indian	2	—	—	—	1	1	3	—	—		[1]
	Total	159	8	0.788 (0.015)	0.426 (0.280)	102**	30	175	8.8	0.686	0.035*	—
Atlantic	Mediterranean	40	1	0.000 (0.000)	0.000 (0.000)	1	8*	9	4.6	0.631	0.086	[1, 7]
	Canary Islands	14	3	0.648 (0.081)	0.329 (0.246)	14	8	25	5.2	0.690	0.014	[1]
	Western North Atlantic	87	6	0.616 (0.028)	0.271 (0.200)	—	—	—	—	—	—	[1, 7]
	Gulf of Mexico	153	5	0.500 (0.044)	0.211 (0.167)	—	—	—	—	—	—	[7]
	Unassigned Atlantic	68	—	—	—	3	0	7	—	—	—	[1, 2]
	Total	362	8	0.748 (0.010)	0.333 (0.231)	18	16	41	8.8	0.669	0.051*	—
	Unassigned Worldwide	41	—	—	—	—	—	—	—	—	—	[2]
	Worldwide total	1587	39	0.818 (0.005)	0.430 (0.279)	319**	143	542	14.0	0.696	0.029	—

A binomial exact test was used to identify areas with a significant bias of females (asterisk after female sample size) or males (asterisk after male sample size), where *significant at $P < 0.05$; **significant at $P < 0.001$. F_{IS} values are indicated as significant where *significant at $P < 0.05$. Regional allelic richness is adjusted by minimum regional sample size, with oceanic allelic richness adjusted by minimum ocean sample size. Regions ordered from east to west. 'Unassigned' includes samples not originating from tropical/subtropical regions, from areas with samples sizes too small to include in regional analyses, or those samples without a specific ~500 km regional location. References for data: [1] This study; [2] Lyrholm & Gyllensten (1998); [3] Rendell *et al.* (2012); [4] Richard *et al.* (1996a); [5] Whitehead *et al.* (1998); [6] Mesnick *et al.* (2011); and [7] Engelhaupt *et al.* (2009).

Because inference of population expansions can be affected by population structure (Ptak & Przeworski 2002; Pannell & Whitlock 2003), we also looked for population expansions at the oceanic and within-ocean regional levels. The Pacific Ocean had a strongly significant negative Fu's *Fs* value ($Fs = -21.5$, $P = 0.0003$), and both the Pacific and Atlantic gave qualitatively unimodal mismatch distributions (Fig. 2), with the population expansion model supported for the Atlantic Ocean ($P > 0.1547$). In contrast, the Indian Ocean showed a multimodal mismatch distribution (Fig. 2) and the Fu's *Fs* value was not significant (Appendix S8, Supporting information). Using estimates of tau for each ocean, the time at expansion within the Pacific was estimated at 66 900 years before present (95% CI: 60 800–87 300); 67 200 years before present in the Atlantic (95% CI: 55 200–86 700) and 94 000 years before present in the Indian Ocean (95% CI: 37 100–150 000). These data suggest a more recent population expansion event in the Pacific, also supported by the large number of within-Pacific regions with negative Fu's *Fs* results (Appendix S8, Supporting information).

mtDNA diversity, differentiation and phylogeography

We resolved a total of 39 mtDNA CR haplotypes in the worldwide data set (Table 1), including twelve previously unreported haplotypes (Fig. 3). Except for *KK*, these new haplotypes were rare ($n < 5$) and only found in one region (Fig. 4). The maximum distance between

Table 2 Summary of microsatellite locus-specific characteristics for the 542 individuals genotyped in this study. *n* gives the number of individuals successfully typed at each locus. H_o and H_e (observed and expected heterozygosity, respectively) calculated in CERVUS. F_{ST} calculated in GENEPOP for oceanic and regional subsets of data (see Table 1)

Locus	*n*	References	Size range (bp)	No of alleles	H_o	H_e	Oceanic F_{ST}	Regional F_{ST}
EV1	521	Valsecchi & Amos (1996)	118–142	12	0.599	0.641	0.0034	0.0061
EV5	529	Valsecchi & Amos (1996)	148–174	11	0.711	0.708	***0.0100****	0.0071
EV14	483	Valsecchi & Amos (1996)	121–155	14	0.687	0.716	0.0032	***0.0120****
EV37	504	Valsecchi & Amos (1996)	177–250	32	0.855	0.905	***0.0029****	***0.0050****
EV94	534	Valsecchi & Amos (1996)	193–225	17	0.82	0.804	0.0017	0.0025
GATA417	438	Palsbøll *et al.* (1997)	172–202	7	0.509	0.532	0.0107	0.0019
GT23	523	Bérubé *et al.* (2000)	75–99	12	0.511	0.499	0.0034	0.0000
GT575	487	Bérubé *et al.* (2000)	131–137	4	0.61	0.611	0.0011	0.0104
rw4-10	461	Waldick *et al.* (1999)	177–213	14	0.72	0.768	***0.0028****	0.0037
SW13	523	Richard *et al.* (1996b)	134–176	14	0.824	0.835	0.0000	***0.0092****
464/465	404	Schlötterer *et al.* (1991)	141–145	3	0.527	0.541	0.0035	0.0000
SW19	508	Richard *et al.* (1996b)	89–167	32	0.88	0.921	***0.0017****	***0.0029*****
FCB1	519	Buchanan *et al.* (1996)	107–145	16	0.792	0.835	0.0018	0.0032
Average	494.9			14.5	0.696	0.717	***0.0032*****	***0.0048*****

Statistically significant F_{ST} values are bolded and italicized, with *significant at $P < 0.05$; **significant at $P < 0.001$.

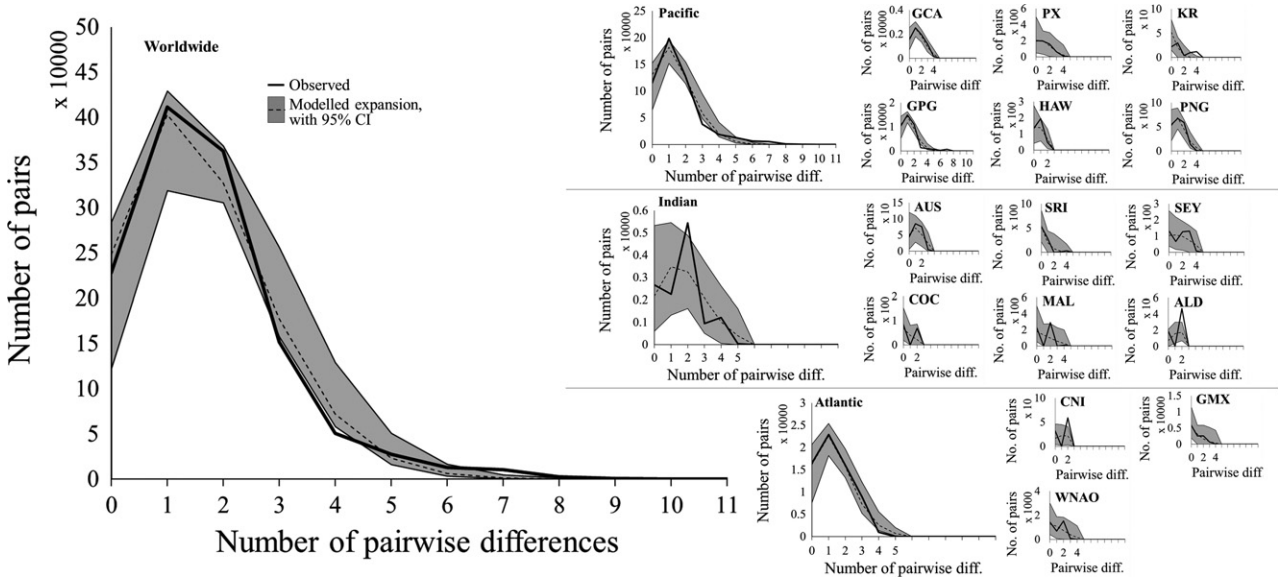


Fig. 2 Mismatch distributions for mtDNA at worldwide, oceanic and regional levels. GCA, Gulf of California; GPG, Galapagos/Ecuador; PX, 'Pacific Crossing'; HAW, Hawai'i; KR, Kiribati; PNG, Papua New Guinea; AUS, SW Australia; COC, Cocos Island; SRI, Sri Lanka; MAL, Maldives/Chagos Archipelago; SEY, Seychelles; ALD, Aldabras; CNI, Canary Island; WNAO, Western North Atlantic; GMX, Gulf of Mexico. A mismatch distribution was not generated for the Mediterranean due to lack of mtDNA variation within this region.

any two haplotypes was two substitutions and this only occurred twice on the haplotype network (Fig. 3). Of the 31 variable sites found over the 394-bp mtDNA CR, all were transitions (Appendix S1, Supporting information). To investigate the potential for resolving further mtDNA diversity, we sequenced 400 samples for 619 bp of the mtDNA CR. A comparison of the two consensus lengths indicated 394 bp captured the majority of variation (Appendix S6, Supporting information). Therefore, even with the addition of Indian Ocean samples, the level of mtDNA CR diversity in the sperm whale is still among the lowest in Cetacea (Table 1 vs. cetacean mtDNA diversity estimates in Alexander *et al.* 2013). The Atlantic Ocean had significantly lower nucleotide diversity than the Indian and Pacific Oceans and significantly lower haplotype

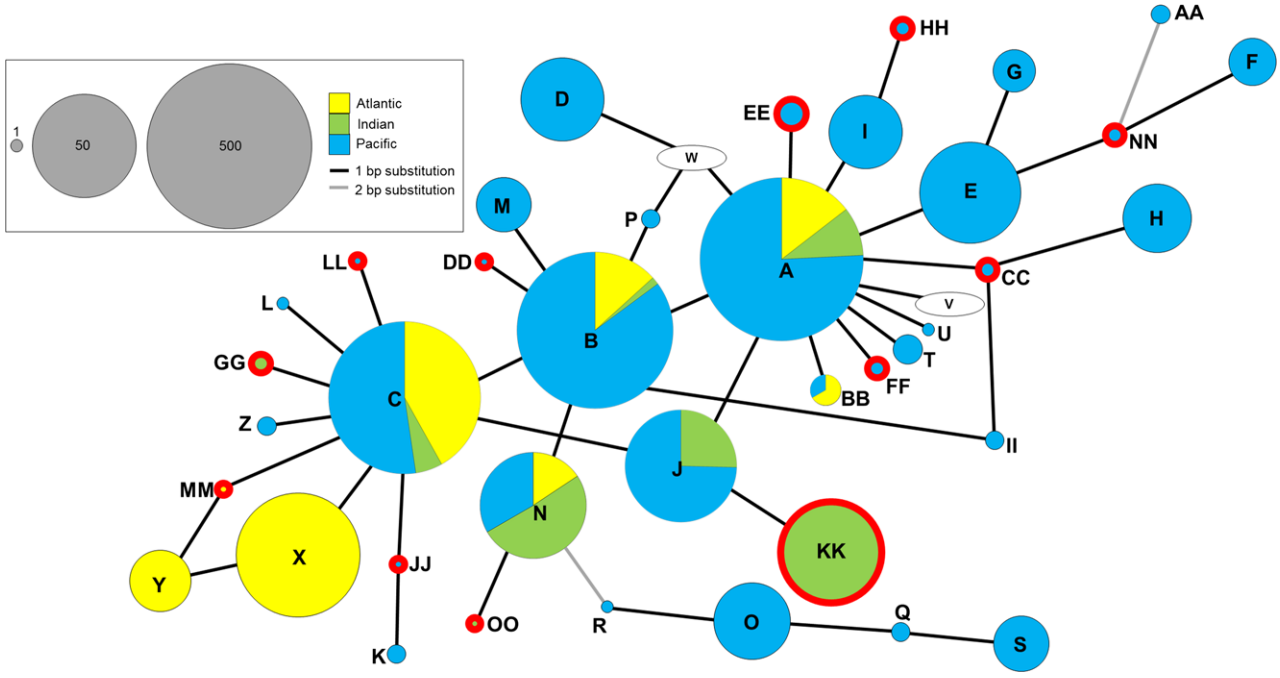


Fig. 3 Maximum parsimony network based on 394 bp of the mtDNA CR (haplotype definitions in Appendix S1, Supporting information). Haplotypes are coloured by ocean, with the exception of haplotypes V and W that were not localized to a specific ocean in previous studies. Size of haplotype pie is proportional on a log scale to the total number of samples with the haplotype. Lines represent substitutions (one or two between haplotypes as defined by the key). New haplotypes characterized in this study are outlined.

diversity than the Pacific (Table 1, *P*-values for all significant diversity comparisons summarized in Appendix S9, Supporting information), which appeared to be partly driven by the lack of mtDNA CR variation within the Mediterranean (Table 1).

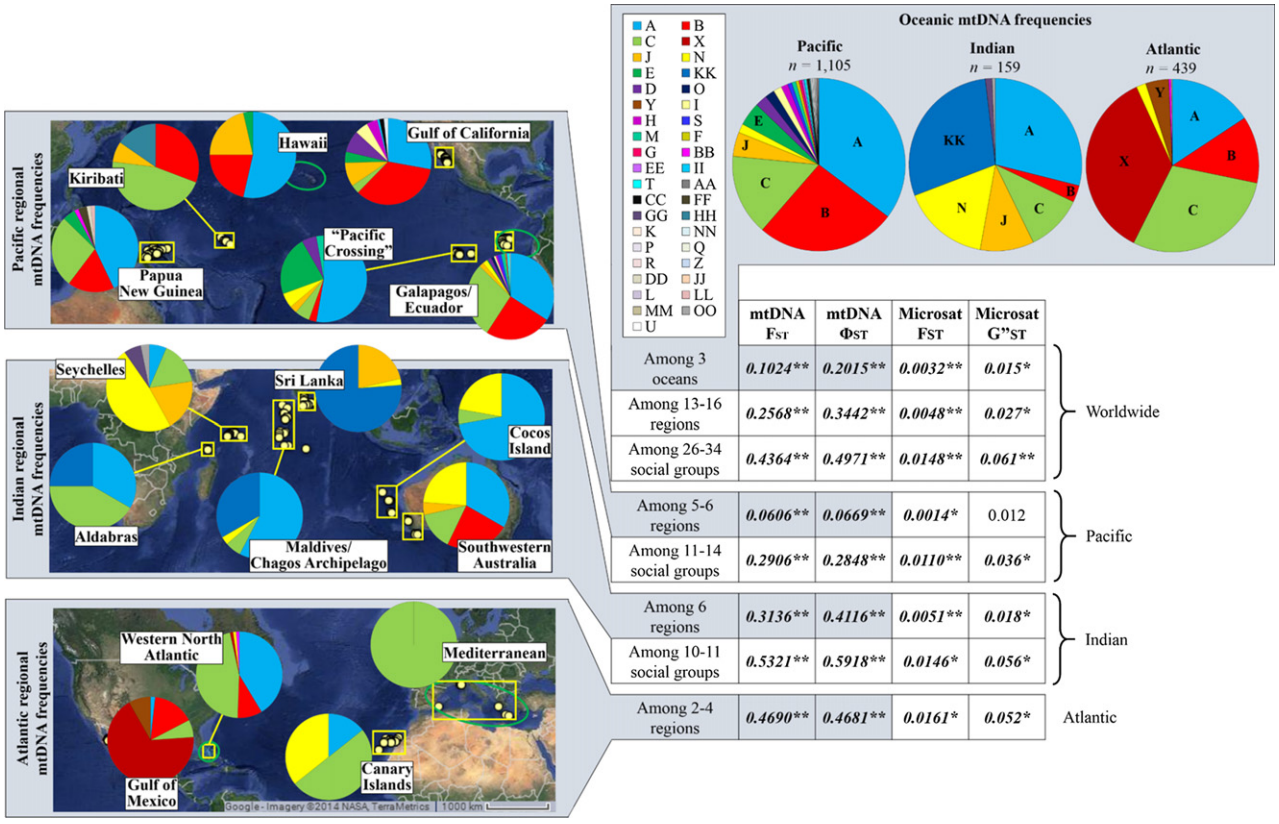
There was some sharing of mtDNA haplotypes across all three ocean basins, particularly of *A*, *B* and *C*: the three most common haplotypes (Figs 3 and 4). Despite this, geographic structure was evident at the oceanic scale, with four haplotypes found at reasonably high frequencies (in >20 individuals) yet restricted to a single ocean basin (haplotype *X* in the Atlantic, *KK* in the Indian Ocean, *E* and *D* in the Pacific Ocean: Figs 3 and 4). Values for F_{ST} and Φ_{ST} showed similar patterns at oceanic and regional levels (Fig. 4, Appendix S10, Supporting information), albeit with Φ_{ST} tending to exceed the magnitude of F_{ST}. An explicit test of these two indices (using N_{ST} as an analog of Φ_{ST}: Pons & Petit 1996) indicated a small but significant ($P < 0.05$) influence of phylogeographic structure at the oceanic level (i.e. haplotype lineages sorted by ocean) and worldwide regional level (i.e. haplotype lineages sorted by region over a worldwide scale). However, these results were contingent on the inclusion of the Gulf of Mexico, which has the closely related haplotypes X and Y present in high frequencies (Figs 3 and 4).

Microsatellite diversity and differentiation

No significant differences in microsatellite heterozygosity or allelic richness were observed between oceans (using Wilcoxon signed rank or *t*-tests depending on the equality of variances and normality of differences between pairs; locus by locus results Appendix S7, Supporting information). Significant differentiation was detected among oceans (F_{ST} 0.003, G''_{ST} 0.015, $P < 0.05$), but this was far lower in magnitude than that observed for mtDNA (Fig. 4). Our STRUCTURE results gave the highest likelihood to $K = 1$ (mean Ln Pr(X|K) = −18 855.3 *cf.* −18 908.9 for $K = 2$). Visual inspection of the structure results for $K = 2$ (Appendix S11, Supporting information) showed no obvious population structure, offering further support for $K = 1$. This is not surprising given the low levels of differentiation found in the a priori analyses partitioning the data set by region (e.g. $F_{ST} < 0.02$, Appendices S10 and S12, Supporting information) (Waples & Gaggiotti 2006).

Evaluating the relative importance of social group vs. geographic regions by ocean

Among the *Odyssey* data set, 67 social groups ($n = 420$ individuals) had more than two individuals pass genetic QC measures. After inclusion of published



	mtDNA F_{ST}	mtDNA Φ_{ST}	Microsat F_{ST}	Microsat G"$_{ST}$	
Among 3 oceans	*0.1024***	*0.2015***	*0.0032***	*0.015**	Worldwide
Among 13-16 regions	*0.2568***	*0.3442***	*0.0048***	*0.027**	
Among 26-34 social groups	*0.4364***	*0.4971***	*0.0148***	*0.061***	
Among 5-6 regions	*0.0606***	*0.0669***	*0.0014**	0.012	Pacific
Among 11-14 social groups	*0.2906***	*0.2848***	*0.0110***	*0.036**	
Among 6 regions	*0.3136***	*0.4116***	*0.0051***	*0.018**	Indian
Among 10-11 social groups	*0.5321***	*0.5918***	*0.0146**	*0.056**	
Among 2-4 regions	*0.4690***	*0.4681***	*0.0161**	*0.052**	Atlantic

Fig. 4 Differentiation of mtDNA and microsatellites at oceanic, regional and social group levels with regional/oceanic mtDNA haplotype frequencies. Where a range in number of social groups/regions are given, the smaller number corresponds to the microsatellite sample size and the larger number to the mtDNA sample size. No social group analysis was conducted for the Atlantic due to limited sample sizes. Braces to right of table give scale of each analysis (worldwide, and by each ocean). See Table 1 for sample sizes used in regional analyses and Appendix S5 (Supporting information) for sample sizes used in social group analyses. Pairwise comparisons at oceanic and regional levels are given in Appendix S12 (Supporting information). Haplotype key ordered by worldwide abundance of haplotype. *significant at $P < 0.05$; **significant at $P < 0.001$.

mtDNA information for 28 social groups from Ecuador and the Gulf of Mexico (Richard *et al.* 1996a; Engelhaupt 2004; Ortega-Ortiz *et al.* 2012), we included 95 social groups ($n = 541$ individuals), representing 16 regions and all 3 oceans (Appendix S5, Supporting information). In non-nested analyses, differentiation among social groups was extremely high: social group consistently explained greater levels of variation than partitioning by regions and oceans (Fig. 4). This is expected, given the more fine-scale partitioning of social groups compared with higher-level geographic scales.

To account for this fine-scale partitioning in a hierarchical AMOVA, we nested social groups with ≥ 5 sampled individuals within ocean and region for the worldwide data set, and within regions for the Pacific and Indian Oceans (the Atlantic did not have enough regions for each genetic marker type with social groups of five or more individuals). For the worldwide data set, social group explained a greater amount of mtDNA variance than either ocean or region, but all levels were significant (Table 3). In the Pacific, only social group (compared with region) explained any significant amount of mtDNA variance (Table 3). In the Indian Ocean, however, region explained a larger percentage of variance than social group, although social group was significant (Table 3). Social group was the only hierarchical level that explained any significant variance in the microsatellite data set (Table 3).

Testing for female philopatry: sex-biased gene flow and dispersal

Most equatorial regions showed a significant skew towards females, consistent with the assumption that the *Odyssey* largely targeted social groups dominated by females (Table 1). Sex-specific estimates of gene flow, calculated from microsatellite and mtDNA (Hedrick *et al.* 2013), were low for females and high for males (Table 4). Given the evidence for sex-biased gene flow, it is not surprising that tests for sex-biased dispersal

Table 3 Degrees of freedom (d.f.) and percentage of variation (%) explained by ocean, region, social group and among individuals for hierarchical AMOVAs, nesting social group within ocean for the worldwide data set, and social group within region for the worldwide, Pacific and Indian Ocean data sets. (a) mtDNA F_{ST}; (b) mtDNA $\Phi_{ST;}$ and (c) microsatellite F_{ST}

	Worldwide				Pacific		Indian	
	d.f.	%	d.f.	%	d.f.	%	d.f.	%
(a) mtDNA F_{ST}								
Among oceans	2	***15.1*****	—	—	—	—	—	—
Among regions	—	—	7	***22.7*****	2	−3.0	3	***44.4*****
Among social groups	31	***32.0*****	22	***26.9*****	9	***34.4*****	6	***12.3*****
Among individuals	350	***52.9*****	292	***50.4*****	162	***68.6*****	90	***43.2****
(b) mtDNA Φ_{ST}								
Among oceans	2	***25.0*****	—	—	—	—	—	—
Among regions	—	—	7	***34.9*****	2	−1.3	3	***51.9*****
Among social groups	31	***29.8*****	22	***21.5*****	9	***31.9*****	6	***10.6****
Among individuals	350	***45.2*****	292	***43.6*****	162	***69.4*****	90	***37.6****
(c) microsatellite F_{ST}								
Among oceans	2	0.27	—	—	—	—	—	—
Among regions	—	—	6	0.07	1	0.03	3	−0.25
Among social groups	23	***1.32*****	15	***1.49*****	7	***1.25*****	7	***1.66*****
Among individuals	281	98.4	222	98.4	110	98.7	104	98.6

Levels which explain a significant percentage of variation are bolded and italicized, with *significant at $P < 0.05$; **significant at $P < 0.001$. The social groups these results are based on are summarized in Appendix S5 (Supporting information).

Table 4 Sex-specific F_{ST} comparisons by marker and estimates of sex-biased gene flow (Nm, $m_M/m_{F,}$ Hedrick *et al*. 2013). Regional analyses of sex-specific F_{ST} were limited to areas with more than two identified females and males as summarized in Table 1. Note that although male-specific microsatellite F_{ST} appears to exceed that of females among regions in the Pacific, neither estimate is significantly different from zero. Due to limited sample sizes, a within-ocean regional F_{ST} analysis was not conducted for the Atlantic. Hedrick *et al*. (2013) estimates of sex-specific gene flow are based on the fixation indices presented in Fig. 4. As all variance of assignment tests (vAIc) were not significant, results of these tests are not shown

		mtDNA CR			Microsatellites			Gene flow	
Area	Sex	*n*	F_{ST}	*P*-value	*n*	F_{ST}	*P*-value	*Nm*	m_M/m_F
Pacific									
By region	F	175	0.1145	0.1632	177	0.0004	0.8438	7.75	22.01
	M	42	0.0640		43	0.0103		170.57	
Indian									
By region	F	70	0.4892	0.1666	83	0.0061	0.4499	1.09	43.56
	M	27	0.2878		29	0.0050		47.68	
Atlantic									
By region	F	—	—	—	—	—	—	0.57	25.99
	M							14.71	
Worldwide									
By region	F	253	0.2735	***0.0366****	274	0.0063	0.2745	1.45	34.82
	M	72	0.1426		80	0.0028		50.39	
By ocean	F	289	0.1259	0.0725	319	0.0068	***0.0351****	4.38	16.77
	M	118	0.0673		143	0.0008		73.49	

Statistically significant values (for the *P*-values for the difference in F_{ST} between sexes) are bolded and italicized, with *significant at $P < 0.05$.

indicated males are the more dispersive sex (Table 4). At all hierarchical levels, female-specific F_{ST} for mtDNA exceeded that of males and was significantly greater when partitioning by regions over the worldwide data set (Table 4). For microsatellites, the magnitude of female-specific F_{ST} was greater than male-specific

differentiation except among regions within the Pacific (however, neither sex's F_{ST} was significantly different from zero). Female-specific microsatellite F_{ST} significantly exceeded that of males at the oceanic scale (Table 4). Surprisingly, the sex-specific variance of assignment tests were not significant.

Discussion

We have shown evidence for multiple forces operating on genetic diversity and differentiation in the sperm whale, a marine species with a worldwide distribution, over different temporal and geographic scales. We suggest the relatively low mtDNA diversity of sperm whales is consistent with a recent population expansion or sweep. However, despite the low mtDNA diversity, we detected marked patterns of maternal structure in the Indian Ocean, similar to that observed in the Atlantic Ocean (this study; Engelhaupt *et al.* 2009), but in the absence of obvious geographic boundaries. In contrast, the Pacific Ocean showed far less regional mtDNA differentiation. Even after accounting for social group in a nested AMOVA, region remained an important level in describing genetic structure within the Indian Ocean, but not the Pacific. This is consistent with previous studies that found no geographically based mtDNA structure in the Pacific (Lyrholm & Gyllensten 1998; Whitehead *et al.* 1998; Lyrholm *et al.* 1999; Rendell *et al.* 2012), or significant, but low levels of differentiation (Mesnick *et al.* 2011), suggesting the Pacific is unusual in its lack of geographic structure in comparison with the Atlantic and Indian Oceans. In contrast with the high levels of maternal structure found at various hierarchical scales, nuclear structure was far less pronounced (albeit significant at the oceanic level, in contrast with previous studies, e.g. Lyrholm *et al.* 1999, likely due to our larger sample sizes). In fact, within the nested AMOVA, social group was the only important level for describing microsatellite variance. Although the lack of nuclear structure could be influenced by the recent population expansion/sweep, it is also likely affected by the presence of male-biased dispersal and gene flow.

A recent worldwide expansion of sperm whales

The mismatch analysis conducted in this study was consistent with a worldwide expansion of a single maternal lineage that began ~80 000 years ago. It is important to point out that this estimate is provisional due to the problems of model fitting and phylogenetically derived substitution rates (Ho *et al.* 2011a; Grant 2015). Indeed, the use of a faster substitution rate derived from ancient DNA sampling (e.g. 20%/million year for bowhead whales: Ho *et al.* 2011b) would lead to an estimate of the expansion beginning ~10 000 years ago, consistent with the end of the last glacial maximum (LGM) (Lambeck *et al.* 2014). In a remarkable parallel, another abyssal predator (and one of the sperm whale's prey), the giant squid (*Architeuthis* spp.), also shows extremely low mitogenomic diversity (Winkelmann *et al.* 2013), and a similar time to most recent common maternal ancestor, depending on the substitution rate used. This raises the possibility that a worldwide expansion of sperm whales could have been predicated on a recent expansion of their prey, especially as other squid species have also shown signatures of demographic/range expansions that appear to be associated with the LGM (e.g. *Dosidicus gigas,* Ibáñez *et al.* 2011; *Doryteuthis gahi*, Ibáñez *et al.* 2012; Ibáñez & Poulin 2014). Further support for this hypothesis comes from other deep-diving, squid-feeding cetaceans which show similar patterns of expansion, including the Gray's beaked whale (*Mesoplodon grayi*) and pilot whales (*Globicephala* spp.) (Oremus *et al.* 2009; Thompson *et al.* 2016). However, as the squid species mentioned are only some of the many cephalopod and fish species preyed on by the sperm whale (Whitehead 2003), future research should establish whether other prey species show the same patterns. This is especially pertinent as other cetacean species with diverse prey bases have also shown signatures of population expansions associated with the LGM (e.g. white-beaked dolphins, Banguera-Hinestroza *et al.* 2010; harbour porpoises, Fontaine *et al.* 2014; killer whales, Morin *et al.* 2015).

In addition, the population expansions of the squid species mentioned could also support the cultural hitchhiking hypothesis, if the use of these squid as prey was restricted to a few initial sperm whale matrilines. The inclusion of population-level nuclear genetic markers in future studies could distinguish between a selective sweep and a population expansion as the cause of low mtDNA diversity. A selective sweep (either due to cultural hitchhiking, or functional selection acting on the mtDNA) will reduce the genetic diversity of the mitogenome, but not of the nuclear genome (Rokas *et al.* 2001; Charlesworth *et al.* 2003). Given the limited phylogeographic structure (i.e. divergence) observed for sperm whale mtDNA, whatever the ultimate cause of the low mtDNA diversity, time since this point has been insufficient for unique ocean-specific or region-specific haplotype lineages (with the exception of the Gulf of Mexico) to be established through mutation and lineage sorting. However, the marked female philopatry present in the sperm whale at regional and social group levels has worked on postexpansion mtDNA diversity to establish strong patterns of mtDNA differentiation within oceans.

Female philopatry at the geographic vs social group level varies by ocean

Levels of regional differentiation in mtDNA were much higher in the Atlantic and Indian Oceans than in the Pacific. Geographic region persisted as an important factor in partitioning mtDNA diversity within the Indian Ocean, even after accounting for social group. In contrast, within the Pacific, social group was the only level that described any significant amount of variation. The lack of regional structure in the Pacific is consistent with behavioural evidence: female whales in the Pacific appear to range further than in the Atlantic, up to ~4000 km in the Pacific and only up to ~700 km in the Atlantic (Jaquet *et al.* 2003; Whitehead *et al.* 2008, 2012; Ortega-Ortiz *et al.* 2012; Mizroch & Rice 2013). Previously, differences in geographic structure and social group composition between the Atlantic and Pacific Oceans have been attributed to oceanography, predation, whaling or culture (Whitehead *et al.* 2012). Our results suggest that a consideration of the factors driving differences in geographic structure should also be extended to the Indian Ocean.

Oceanography can influence differentiation through geographic isolation (e.g. the Mediterranean, Gulf of Mexico: Engelhaupt *et al.* 2009). However, geographic isolation cannot explain the large degree of mtDNA differentiation observed within the Indian Ocean, particularly illustrated by the mtDNA haplotype frequency differences in comparison with Sri Lanka. Instead, a potential oceanographic explanation lies in the bathymetry of Sri Lanka: there are a large number of submarine canyons that lead to enhanced productivity of this region (de Vos *et al.* 2012). Female sperm whales utilizing the Sri Lankan canyons might not need to range as widely to satisfy nutritional requirements (Gordon 1987; Moors-Murphy 2014). This 'enhanced philopatry' could then lead to the striking geographic differentiation in mtDNA observed. A similar process of local fidelity has been proposed for insular communities of otherwise pelagic dolphins, due to an 'island mass' effect (Martien *et al.* 2012; Oremus *et al.* 2012).

Another potential explanation for the difference in geographic structure between oceans lies in the acoustic culture of sperm whales. It has been previously hypothesized that acoustic clans, which comprise of social groups with similar repertoires of acoustic codas (stereotypical series of clicks), shape patterns of genetic differentiation in the sperm whale (Watkins & Schevill 1977; Whitehead *et al.* 1998; Rendell *et al.* 2012). In the Atlantic, coda patterns vary based on geographic regions and acoustic clans are allopatric (Whitehead *et al.* 2012). This correlates with the heightened patterns of geographically based mtDNA differentiation observed in this ocean. In the Pacific, acoustic clans are distributed sympatrically across broad geographic ranges (Whitehead *et al.* 1998; Rendell *et al.* 2012; Cantor *et al.* 2015). It has been previously proposed that the lack of geographically based mtDNA differentiation in the Pacific is because maternal dispersal and gene flow occur within acoustic clans, but across broad geographic scales (i.e. females are socially philopatric rather than geographically philopatric; Whitehead *et al.* 1998; Rendell *et al.* 2012; Cantor *et al.* 2015). This hypothesis is consistent with our nested AMOVA results for the Pacific, where social group was the only level that explained any significant amount of genetic variation. However, genetic structure driven by oceanography or culture are not necessarily mutually exclusive hypotheses: differences in resource use could be reinforced by differences in coda repertoire between acoustic clans (Cantor & Whitehead 2015; Cantor *et al.* 2015; Gero *et al.* 2015).

Local population declines due to whaling could have also reduced geographic structure in the Pacific (Whitehead *et al.* 2012). Large-scale movements from the Galapagos to Peru have been documented, where whaling-related declines left the productive Humboldt Current underpopulated (Whitehead *et al.* 1997). Indeed, the Pacific, particularly the North Pacific, was subjected to high levels of both legal and illegal whaling (Ivashchenko *et al.* 2013). Alternately, perhaps there has been insufficient time in the Pacific for geographic structure to evolve, for either genetic diversity or vocalization patterns, given our results suggested a more recent expansion in the Pacific. Distinguishing between whaling and pre-human causes of the Pacific-wide expansion will require additional genetic data to establish tighter confidence intervals on the relative timing of population expansions between the oceans. Whether the signature of a population expansion has been exacerbated by whaling or not, an expansion would be expected to tightly correlate both the maternally inherited mtDNA and maternally influenced coda type (Whitehead *et al.* 2012; Cantor *et al.* 2015). This could be further tested using linked acoustic and genetic sampling in the Indian Ocean (e.g. Rendell *et al.* 2012). We would predict that in the Indian Ocean, mtDNA genetic variation would be at equilibrium with both coda and geographic structure, as it is in the Atlantic.

Female philopatry and male-biased dispersal

Our findings confirm the importance of female philopatry and male-biased dispersal in the sperm whale (Lyrholm *et al.* 1999; Engelhaupt *et al.* 2009). In addition to sex-biased dispersal, we demonstrated that the sperm whale shows male-biased gene flow. Male-biased gene flow could explain the significant among-group

microsatellite differentiation detected in this study: differing paternal contributions to the alleles present within each female social group would enhance nuclear genetic drift between social groups (Richard *et al.* 1996a). This mechanism could be investigated in the future using a gametic mark–recapture framework to detect paternities among different social groups (Garrigue *et al.* 2004; Carroll *et al.* 2012). However, despite the overall patterns of male-biased dispersal and gene flow, significant microsatellite differentiation between oceans indicates some restriction in oceanic dispersal and gene flow, even of males. There is also some evidence for breeding fidelity of males at even finer spatial scales than at the oceanic level, as suggested by a possible first-order kinship between two males in the Chagos Archipelago. Evidence for male fidelity has also been found in the Californian Current by Mesnick *et al.* (2011) using genetic assignment. These findings could indicate that sex-biased dispersal in sperm whales is facultative rather than obligate; for example, some males show philopatry to specific areas, while others disperse. A re-examination of other species (e.g. great white sharks, Pardini *et al.* 2001; humpback whales, Baker *et al.* 2013) that show apparent signatures of male-biased gene flow could be of interest to establish whether this phenomenon is found in other taxa.

Management implications

Sex-biased dispersal and strong maternal population structure in the sperm whale argue for management units based on the more philopatric females, rather than the wider ranging males, requiring female-specific estimates of population size similar to male-specific effective population size estimates in humpbacks (Constantine *et al.* 2012). In addition, when defining female-based population structure in the sperm whale, it is important to aggregate samples at appropriate spatial scales (Donovan 1991; Dufault *et al.* 1999). Given the clustered sample collection of the *Odyssey*, we chose to group samples that occurred within 500 km of another sample. This could have inadvertently either split regions that were truly one population, or alternately 'lumped' areas with more than one distinct population. Both of these alternatives present problems. 'Splitting' regional populations could mean that the strong differences between social groups detected in our current research, as well as previous studies (Lyrholm & Gyllensten 1998; Rendell *et al.* 2012), are conflated with regional differentiation. The alternative of 'clumping' can also be problematic as regions could represent areas of different importance for males and females (e.g. the Maldives/Chagos Archipelago region, where only males were sampled around the Chagos Archipelago, but both sexes around the Maldives). Although we attempted to assess these a priori divisions against a non-a priori clustering method (STRUCTURE), this was limited by low levels of differentiation and the relatively small number of microsatellite loci.

Continuing to investigate patterns of genetic differentiation in the sperm whale is important, as high degrees of differentiation (i.e. isolation) could indicate susceptibility to population declines resulting from various ongoing anthropogenic threats, including: entanglement in marine debris and ship strike (Notarbartolo-Di-Sciara 2014); exposure to pollutants (Wise *et al.* 2009, 2011; Savery *et al.* 2013a,b), as well as pollution associated with oil extraction (e.g. 2010 *Deepwater Horizon* oil spill) and negative interactions with anthropogenic sound (Mate *et al.* 1994).

Conclusion

In this study, we demonstrated that low mtDNA diversity in the sperm whale is likely due to a recent population expansion. Despite low mtDNA diversity, we demonstrated high levels of regional structure within some ocean basins. However, social group was also an important level in describing mtDNA variance. The importance of social group and geographic philopatry differed by ocean, with only social group explaining any significant amount of mtDNA variance in the Pacific Ocean. Being able to distinguish between geographic and social group philopatry is important as a restriction in movement between local populations could indicate that there is a real risk of long-term declines in response to current anthropogenic threats, despite the sperm whale's large worldwide population size. The approach we have used in this study for partitioning the effects of social group and geographic regions will also be useful for other species that show strong social structure, yet are of conservation concern, such as elephants, the long-finned pilot whale and the killer whale (Ottensmeyer & Whitehead 2003; Hoelzel *et al.* 2007; Archie *et al.* 2008).

The specific mechanism(s) driving the differing contributions to genetic structure within oceans requires further study: particularly whether the expansion within the Pacific is more recent than in other oceans and how acoustic codas are structured in the Indian Ocean. However, overall, the high levels of mtDNA structure observed in the sperm whale appear to be driven by female philopatry at multiple hierarchical levels, contrasting with male-biased dispersal and gene flow. By investigating the interplay of evolutionary forces operating at different temporal and geographic scales, we have shown that sperm whales are perhaps a unique example of a global population expansion followed by rapid assortment due to female social organization.

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A.A. performed genetic analyses, wrote analysis scripts, analysed data and wrote the paper. D.S. and K.H. performed genetic analyses and analysed data. S.L.M. and

D.E. provided analysis recommendations. I.K. and R.P. designed the *Odyssey* sample collection strategy, performed field research and collected field data. C.S.B. supervised the research design and provided analysis recommendations. All authors provided editorial input to writing of the paper.

Data accessibility

For each sample genotyped in this study, general location (as well as latitude/longitude where available), individual ID code, social group code, sex, microsatellite genotype and a letter code denoting the mtDNA CR haplotype (defined in Appendix S1, Supporting information) are archived with Dryad (doi: 10.5061/dryad.2q4r0).

Sequences of each defined haplotype have been archived on NCBI GenBank (Accession nos: KU719571–KU719622).

Scripts used in analyses are available with online Supplementary Materials or at https://github.com/laninsky/genetic_diversity_diffs.

Supporting information

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

Appendix S1 Summary of the variable sites that define the sperm whale mtDNA CR haplotypes found in this study.

Appendix S2 Summary of the sperm whale mtDNA CR haplotypes obtained for samples from previous publications, with ocean and geographic region.

Appendix S3 PCR reaction conditions, dye-labels, repeat unit, size range, and reference for the 13 microsatellite loci used in this study.

Appendix S4 R code for comparing microsatellite heterozygosity/allelic richness levels between different geographic partitions.

Appendix S5 Social groups used in genetic analyses, separated by headers indicating region and ocean.

Appendix S6: 619 bp mtDNA CR analyses of diversity and differentiation.

Appendix S7 Diversity results by microsatellite locus and for the 'kin-restricted' dataset.

Appendix S8 Population expansion tests for mtDNA (Fu's *Fs*, mismatch distribution fit as assessed through SSD and Raggedness r values, as well as the inferred expansion timing in years before present, and 95% CI).

Appendix S9 Significance of comparisons of haplotype and nucleotide diversity values at various geographic levels.

Appendix S10 Summary of circumequatorial regional differentiation of mtDNA (F_{ST} and Φ_{ST}) and microsatellites (F_{ST} and G''_{ST}) for sperm whales.

Appendix S11 Structure plot (K = 2) based on 13 microsatellites for the *Odyssey* samples where explicit spatial coordinates were available.

Appendix S12 Pairwise comparisons of differentiation for mtDNA and microsatellites.