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Female philopatry in coastal basins and male dispersion across the North Atlantic in a highly mobile marine species, the sperm whale (*Physeter macrocephalus*)

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Abstract

The mechanisms that determine population structure in highly mobile marine species are poorly understood, but useful towards understanding the evolution of diversity, and essential for effective conservation and management. In this study, we compare putative sperm whale populations located in the Gulf of Mexico, western North Atlantic, Mediterranean Sea and North Sea using mtDNA control region sequence data and 16 polymorphic microsatellite loci. The Gulf of Mexico, western North Atlantic and North Sea populations each possessed similar low levels of haplotype and nucleotide diversity at the mtDNA locus, while the Mediterranean Sea population showed no detectable mtDNA diversity. Mitochondrial DNA results showed significant differentiation between all populations, while microsatellites showed significant differentiation only for comparisons with the Mediterranean Sea, and at a much lower level than seen for mtDNA. Samples from either side of the North Atlantic in coastal waters showed no differentiation for mtDNA, while North Atlantic samples from just outside the Gulf of Mexico (the western North Atlantic sample) were highly differentiated from samples within the Gulf at this locus. Our analyses indicate a previously unknown fidelity of females to coastal basins either side of the North Atlantic, and suggest the movement of males among these populations for breeding.

Keywords: cetacean, microsatellite DNA, mitochondrial DNA, philopatry, *Physeter macrocephalus*, sperm whale

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Introduction

Vicariance, isolation across physical barriers and isolation by distance are all common mechanisms for the generation of population genetic structure and speciation. In the marine environment, within an ocean basin, physical barriers are less obvious than in terrestrial habitats, but can include oceanic currents or thermal fronts [e.g. differentiation across the Almeria-Oran front in the Mediterranean Sea (MED); Naciri *et al.* 1999; Perez-Losada *et al.* 2002]. Isolation by distance is sometimes determined by distance along oceanic currents for species with larval drift (as opposed to direct

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line geographical distance; e.g. Knutsen *et al.* 2007), or disrupted by local habitat dependence (e.g. Natoli *et al.* 2005). Marine mammals are highly mobile and possess the ability to move over large distances (e.g. Stevick *et al.* 2002). However, some move for foraging over much broader ranges than for breeding, and there can be substantial differences in the movement patterns of males and females (see Hoelzel 2008). For example, the southern elephant seal (*Mirounga leonina*) travels thousands of kilometres on foraging excursions (Biuw *et al.* 2007), and males disperse great distances for breeding (Fabiani *et al.* 2003), but females are philopatric to breeding sites over much smaller geographical ranges (Fabiani *et al.* 2003). A similar pattern of male and female dispersion was described for the great white shark (*Carcharodon carcharias*; Pardini *et al.* 2001). Regional killer whale (*Orcinus orca*) populations show unique, fixed mtDNA haplotypes, indicating a lack of female movement, while ongoing male-mediated dispersal was detected at a low level for both proximate and distant populations in the North Pacific (Hoelzel *et al.* 2007).

The extent of social structure (e.g. matrilineal based groups) and resource specialization may play a key role in the structuring of cetacean populations (e.g. Hoelzel *et al.* 1998; Whitehead 1998; Hoelzel 2008). For example, strong matrifocal social groups in the killer whale probably determine the extreme pattern of female philopatry seen for some populations (e.g. Hoelzel *et al.* 2007), and habitat boundaries apparently define five bottlenose dolphin (*Tursiops truncatus*) populations between the Black Sea and Scotland (Natoli *et al.* 2005). Female philopatry and male dispersal are the expected patterns of dispersion for mammalian species based on the expectation that partuating females will be more dependent on local resources (Greenwood 1980).

Here we investigate a species that is well known for both matrifocal social behaviour and long-range movement (see Whitehead & Weilgart 2000). Sperm whales are cosmopolitan in distribution (Rice 1989a), rivalled in this respect among odontocetes (toothed whales) only by killer whales (*Orcinus orca*). They exhibit the greatest degree of sexual dimorphism among cetaceans (Best 1979; Rice 1989a). Physically mature males typically range over large distances on their own (Best 1979; Rice 1989a; Whitehead 1993; Whitehead & Weilgart 2000), and are the predominant age–sex class found in high-latitude environments (Whitehead 2003). Females and younger males are predominantly found in mixed sex social groups and units, although young males sometimes form loose aggregations called bachelor groups (Best 1979; Whitehead & Arnbom 1987; Childerhouse *et al.* 1995; Lyrholm & Gyllensten 1998; Lettevall *et al.* 2002).

Various types of data including dialects, mark–recapture data, morphology, parasitism and predation (Best 1979; Whitehead 1987; Whitehead & Arnbom 1987; Arnbom & Whitehead 1989; Rice 1989a; Whitehead & Kahn 1992; Dufault & Whitehead 1998; Whitehead *et al.* 1998) have suggested philopatry among female sperm whales, especially for comparisons among oceans. Recent studies have indicated very low levels of mtDNA nucleotide variation on a global scale, and microsatellite DNA analyses indicate significant levels of kinship between some group members, believed to be the result of matrilineal structuring at the unit or group level (Lyrholm *et al.* 1996, 1999; Richard *et al.* 1996*a*; Christal 1998; Lyrholm & Gyllensten 1998; Whitehead *et al.* 1998; Bond 1999). Although the level of mtDNA genetic structure between global populations was low, there were statistically significant patterns of differentiation between oceans (Lyrholm & Gyllensten 1998). By comparison, studies examining nuclear microsatellite DNA revealed either no significant (Lyrholm *et al.* 1999) or low, but significant (Bond 1999) degrees of population structuring between oceans. In addition, the same studies failed to detect any differentiation for smaller scale geographical comparisons within either the North Pacific or North Atlantic Oceans. These authors suggested that the discrepancy between mtDNA and microsatellite DNA differentiation may reflect sex biased dispersal, with females as the more philopatric sex, consistent with the various earlier studies using mark–recapture, acoustics and environmental markers. Drouot *et al.* (2004) found some differentiation for mtDNA comparing sperm whales from within and outside the MED.

Our study focuses on the role of geographical scale and behaviour in a highly social species with a tremendous potential for long-range dispersion. We studied population structure within the North Atlantic, and considered putative populations in major basins on either side of the Atlantic: the Gulf of Mexico (GOM) and the MED. Year-round sightings and re-sightings of individual whales over periods of days to years from numerous distribution and abundance surveys and research cruises in the northern GOM and the MED suggested that some sperm whales exhibit a degree of philopatry to these geographical areas (Davis *et al.* 1998; Weller *et al.* 2000; Waring *et al.* 2001; Drouot-Dulau & Gannier 2007; A. Frantzis, personal observations). We investigated this possibility by comparing these basins with samples from other North Atlantic regions. We test the hypothesis that these coastal basins represent isolated gene pools of matrifocal social units. We further test the hypothesis that male-mediated gene flow connects these geographically isolated regions on an oceanic scale.

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Materials and methods

Sample collection, preservation and DNA extraction

In total, 301 sperm whale tissue samples collected from four geographical regions [GOM, $n = 153$; Western North Atlantic Ocean (WNAO), $n = 84$; MED, $n = 44$; North Sea (NSEA), $n = 20$] were analysed in this study (Fig. 1; Table 1). Free-ranging GOM samples were collected between May and September during the years 2000–2005 and during the spring of 2001 (Dry Tortugas area only). MED samples from free-ranging whales were collected during the summer months of June–September 2000–2004. WNAO samples from free-ranging whales in the Caribbean were collected between January and May 2005 and from further northeast in the WNAO (Fig. 1) between May and June 2004. Tissue samples were collected from free-ranging sperm whales using biopsy and sloughed skin retrieval techniques (Lambertsen 1987; Palsbøll *et al.* 1991; Amos *et al.* 1992), including sloughed skin opportunistically collected from suction cups that had been attached to sperm whales (Miller *et al.* 2004). Note that recovery of DNA from sloughed skin is somewhat unpredictable, and this affected the number of amplifications possible for some loci (Table 1). Biopsy samples were collected from free-ranging whales using a sterilized corer tip attached to a dart (with float) fired from a crossbow or PAXARMS biopsy system, and stored in salt/DMSO (Amos & Hoelzel 1991). Archived material was also obtained from stranded whales in each region, which made up 100% of the NSEA samples. Specifically, the NSEA samples came from the Orkney coast: ($n = 11$) stranded in 1994 (all individuals stranded in the group were sampled), the Grampian coast: ($n = 6$) stranded in 1996 (all individuals stranded in the group were sampled), and from three additional strandings in 1993, 1995 and 1998, one sample from each. Whole-cell DNA was extracted by a standard phenol/chloroform method.

Gender determination

Sex was determined using the ZFX/ZFY technique described by Bérubé & Palsbøll (1996). Male and female strandings of known gender from the GOM and NSEA were included as a means of positive confirmation for PCR amplifications.

Microsatellite analysis

Sixteen polymorphic microsatellite loci [EV1, EV5, EV37, EV94, EV104 (Valsecchi & Amos 1996); SW10, SW13, SW19 (Richard *et al.* 1996*b*); FCB1, FCB14, FCB17 (Buchanan *et al.* 1996); DO8, D22 (Shinohara *et al.* 1997); GATA28, GATA417 (Palsbøll *et al.* 1997); TEXVET5 (Rooney *et al.* 1999)] were amplified in separate 15 μL PCR reactions with the following conditions: 100 mM Tris-HCl, pH 8.4, 500 mM KCl, 1.5–2.0 mM $MgCl_2$, 200 μM of each dNTP, 0.25 μM of each primer, 0.025 μM of a labelled primer, 0.3 units of Taq and 10–100 ng of template DNA. Two thermocycling profiles

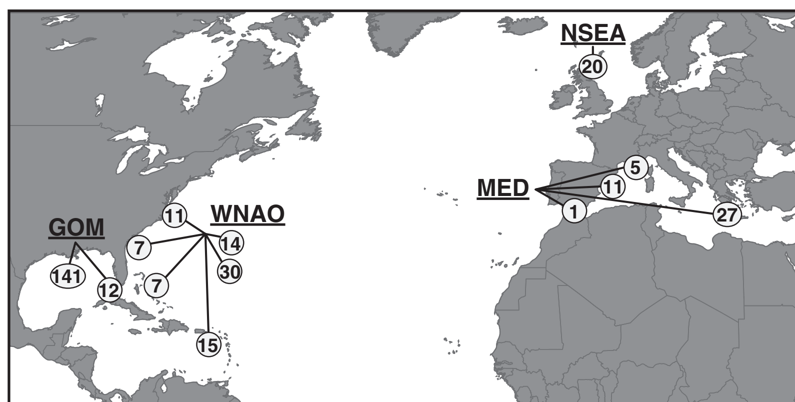

Fig. 1 Locations of sampled whales; abbreviations are defined in Table 1.

Table 1 Analysed populations, abbreviations and the number of samples included in mtDNA and microsatellite analyses

Population	Abbreviation	mtDNA 'all'	mtDNA 'restricted'	msatDNA 'all'	msatDNA 'restricted'
Gulf of Mexico	GOM	153	40	83	40
Mediterranean Sea	MED	38	20	44	22
North Sea	NSEA	18	16	20	18
Western North Atlantic Ocean	WNAO	84	31	66	31

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(denoted as PM1 and PM2) were utilized for all loci tested. The PM1 profile consisted of an initial denaturing step of 95 °C for 5 min, 35 cycles (30 s at 95 °C, 60 s at specified annealing temperature, 60 s at 72 °C) followed by a final extension step of 8 min at 72 °C. The following 13 loci and their respective annealing temperatures were run in the PM1 profile: EV1: 57 °C, EV5: 59 °C, EV94: 55 °C, EV104: 54 °C, SW10: 56 °C, SW13: 57 °C, SW19: 56 °C, D22: 59 °C, FCB1: 53 °C, FCB14: 53 °C, FCB17: 56 °C, GATA28: 53 °C and GATA417: 56 °C. The PM2 profile consisted of an initial denaturing step of 95 °C for 5 min, 35 cycles (60 s at specified annealing temperature, 60 s at 72 °C, 40 s at 94 °C) followed by one cycle at the specified annealing temperature and a final extension step of 5 min at 72 °C. The following three loci and their respective annealing temperatures were run in the PM2 profile: EV37: 56 °C, D08: 52 °C and TEXVET5: 60 °C. Amplified DNA was analysed for length variation on 6% polyacrylamide denaturing gels using fluorescent imaging on an automated ABI PRISM 377 (Applied Biosystems). Results were then imported into the program Genotyper 2.0™ so that allele sizes could be visualized and scored. Paetkau & Strobeck's (1994) probability of identity method was utilized to provide assurance that duplicate samples have been removed. After the elimination of duplicate samples, individuals from the GOM (n = 83), MED (n = 44) and the NSEA (n = 20) were screened for all 16 polymorphic microsatellite loci, while the WNAO sample set (n = 66) was screened across 13 loci (excluding D08, D22 and SW13) (Table 1). WNAO samples were run in a separate laboratory (Northwoods DNA Laboratories), together with 26 control samples to test for differences among the two laboratories (consistent differences found and corrected for; data not shown).

The number of unique alleles was calculated for each locus and over all loci and the observed heterozygosity (H_O) and expected heterozygosity (H_E) levels at each locus were calculated using the computer program Cervus 2.0 (Marshall *et al.* 1998). Deviation from HWE using the Fisher's exact test was performed in the Genepop 3.2a program (Guo & Thompson 1992) using 1000 dememorizations, 1000 batches and 1000 iterations, applying the sequential Bonferonni correction (Rice 1989b). The frequency of putative null alleles, F_{IS} and allelic richness for each locus/population was tested using the computer program FSTAT 2.9.3.2 (Goudet 2001). A test for linkage disequilibrium (null hypothesis: independence between genotypes at separate loci) was completed for each pair of loci in the Genepop 3.2a program by implementing the Fisher's exact test and the Markov chain method (1000 dememorizations, 1000 batches and 1000 iterations per batch), applying the sequential Bonferonni correction.

Comparisons of microsatellite allele frequency distributions at each locus and between geographical populations were evaluated with the Fisher's exact test (Raymond & Rousset 1995) using the population differentiation method in the program Genepop 3.2a. Estimates of Wright's fixation index, F_{ST} (Wright 1951; Weir & Cockerham 1984), were calculated using ARLEQUIN 3.1 and FSTAT 2.9.3.2 computer programs. Rho_{ST}, which is based on the stepwise mutation model (SMM), was calculated using permutation tests with 1000 iterations in the program RSTCALC 2.2 (Goodman 1997). The program STRUCTURE v. 2.2 (Pritchard *et al.* 2000) was used to assign individuals to K populations based on their multilocus genotypes. Admixture and correlated allele frequencies were assumed with 500 000 burn-in steps, 1 000 000 Markov Chain Monte Carlo repetitions and three replicates for each K (assessed for K = 1–4). This was based on 13 loci to include all population samples, but separate runs based on three populations and 16 loci gave comparable results (data not shown).

An assessment as to whether differences in female and male dispersal rates affect population structure with respect to co-dominant genetic markers was performed using the program FSTAT 2.9.3.2 (Goudet *et al.* 2002). This was based on comparisons between both sexes for FIS, FST, HO, HS (the within group gene diversity), mean assignment and assignment variance. Weir & Cockerham's (1984) estimator of F_{IS} and F_{ST} was used. Testing assumes that the species in question has nonoverlapping generations where dispersal occurs at the juvenile stage (before reproduction) and that an individual is sampled postdispersal (Goudet *et al.* 2002). When comparing allele frequencies between individuals of the dispersing sex and those of the more philopatric sex, a greater similarity is expected among the more dispersing sex (Goudet *et al.* 2002). These tests were performed only on those samples run for 16 microsatellite loci to maximize power.

The possibility of population bottlenecks was assessed using the program Bottleneck 1.2.02 (Cornuet & Luikart 1996). Significance of heterozygote excess was assessed using the sign test and Wilcoxon test, as implemented in the program, and based on 1000 replicates. Results are reported for the infinite allele model (IAM), two-phase mutation model (TPM; variance = 30%, probability = 70%) and the SMM. The mode-shift distribution was also assessed for deviation from the normal L-shape as an indicator of a recent bottleneck (see Cornuet & Luikart 1996).

mtDNA analysis

The first 399 bp at the 5′-end of the mtDNA control region was amplified for 293 samples (Table 1) using

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primers L15812 5′-CCTCCCTAAGACTCAAGG-3′ (Arnason *et al.* 1993) and H16343 5′-CCTGAGAATGCA-ACTAGAGG-3′ (Southern *et al.* 1988) in separate 30 μL PCR reactions with the following conditions: 100 mM Tris-HCl, pH 8.4, 500 mM KCl, 1.3 mM $MgCl_2$, 200 μM of each dNTP, 0.25 μM of each primer, 0.3 units of Taq polymerase and 10–100 ng of template DNA. mtDNA sequence data for the additional individual whales from the GOM (n = 70) and the WNAO (n = 84) were from the work performed at Northwoods DNA Laboratories. The PCR thermocycling profile consisted of an initial denaturing step of 95 °C for 5 min, 35 cycles (90 s at 55 °C, 90 s at 72 °C, 45 s at 72 °C), followed by one cycle of 55 °C for 90 s, and a final extension step of 8 min at 72 °C. Purified PCR product was sequenced directly using the ABI 377 and BigDye sequencing kit (Applied Biosystems). Sequences were automatically aligned and then edited by eye using the Sequencher 4.2.1 software (Gene Codes Corp., Ann Arbor, MI, USA). Both strands (forward and reverse) were sequenced for unique haplotypes as a means of polymorphic site confirmation.

Standard measures of diversity including haplotype frequencies, haplotype and nucleotide diversity (h and π respectively; Nei & Chesser 1983) and the number of sequence polymorphic sites were calculated for all samples and for each putative population using ARLEQUIN 3.1 software. Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) statistics were also calculated using ARLEQUIN 3.1 to assess the selective neutrality of the locus. These tests are based on the infinite-site model without recombination, which is suitable for short mtDNA sequences (Schneider *et al.* 2000).

The divergence between populations was assessed using exact tests, conventional F-statistics (F_{ST}) and Φ_{ST} statistical measures carried out using ARLEQUIN 3.1 software (Schneider *et al.* 2000). Estimates of Φ_{ST} used the Tamura–Nei genetic distance model (Tamura & Nei 1993) with a gamma distribution correction value of $\alpha = 0.47$ (Wakeley 1993). A range of $\alpha = 0.30$–0.60 was also tested, with no differences found. The exact test was performed using 100 000 Markov chain steps for increased statistical significance. F_{ST} and Φ_{ST} values were tested for statistical significance via 10 000 permutations of the data.

Controlling for possible kinship bias in sampling

To control for potential kin-sampling bias for population comparisons using mtDNA and microsatellite DNA loci, two data sets deemed 'all' and 'restricted' were used. The *restricted* population structure estimates were performed using a combination of the programs Kinbegone 1.3.1 (B. Taylor and S. Mesnick, unpublished data) and Relatedness 5.0.7 (Goodnight Software, Rice University, Houston, Texas, USA) to eliminate highly related whales from the samples. To remove relatives, Kinbegone uses nuclear genetic markers to first estimate pairwise relatedness and then sequentially remove relatives, until no remaining individuals are related at greater than a given threshold ($R > 0.30$ in our study) (see Engelhaupt 2004 for additional details). Analysis of molecular variance (AMOVA) (run in ARLEQUIN) was used to assess further possible kin clustering both for the restricted and un-restricted data sets. Samples incorporated in the 'all' and 'restricted' data sets for each population are provided in Table 1. The 'restricted' data sets were 'pruned' to eliminate close kin.

Results

Gender Determination

In the GOM, gender was determined for 149 of the 153 individuals tested providing a sex ratio of females to males of 2.55:1 (0.718:0.282), which is significantly different than an expected ratio of 1:1 ($\chi^2 = 28.36$, $P < 0.001$). None of the males that were sampled was clearly physically or sexually mature based on approximate length estimates. However, several of these 'young' males may be either in or nearing sexual maturity based on Best's (1979) sexual maturity estimates. For example, one male was measured at 12.4 m length using photogrammetry (Miller *et al.* 2004). Gender results were obtained for 39 of the 44 MED whales tested. The MED's sex ratio of females to males was 0.50:1 (0.333:0.667), which is significantly different from an expected ratio of 1:1 ($\chi^2 = 4.333$, $P < 0.05$). This may result from sampling bias, as individual males were more readily sampled (from sloughed skin) than females in groups at this location. The 20 samples collected from NSEA stranding events were all from males ranging in size from 12 to 15 m in length (mean = 12.96 m, SE = 0.166). In the WNAO, gender was determined for 58 of the 66 individuals tested providing a sex ratio of females to males of 1.90:1 (0.655:0.345), which is significantly different from an expected ratio of 1:1 ($\chi^2 = 5.586$, $P < 0.05$).

mtDNA sequence results

The first 399 bp of the 5′ mtDNA control region from this study was compared among 293 individual sperm whale samples from the GOM, MED, NSEA and WNAO, Six (1.5%) polymorphic nucleotide sites defining a total of only seven unique haplotypes were found between the populations (GenBank accession numbers: DQ512921-23, DQ512934, DQ512944, DQ512945, DQ512948). All nucleotide substitutions

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between haplotypes were transitions. Shared haplotypes, distribution of haplotypes and haplotype frequencies are provided in Table 2. One haplotype (Y) was unique to the GOM, two haplotypes (N and BB) were unique to the WNAO, while all samples collected in the MED population shared the same 'C' haplotype; one that can be found throughout each of the three geographical regions.

Haplotype diversity (*h*), nucleotide diversity (π) and the mean number of pairwise differences for each individual population were low for the GOM, NSEA and WNAO data sets and invariant for the MED (Table 3). The results of Tajima's *D* for each 'all' and 'restricted' population in the GOM, NSEA and WNAO were non-significant ($P > 0.05$). Fu's F_S test confirmed these results ($P > 0.10$ for each population). Although the resolution was low because of the small number of haplotypes present in the sample set, the mismatch distribution tests showed no significant deviation from the unimodal model that suggests expansion (data not shown).

Genetic differentiation among pairwise populations was tested using an exact test, AMOVA, F_{ST} and Φ_{ST}. The exact test of population subdivision revealed significant differentiation between all populations for comparisons carried out for both the ''all'' and ''restricted'' data sets (*P*-value <0.0001), except for populations in the North Atlantic Ocean (Table 4). AMOVA results (based on Φ_{ST}) indicate that 53.91% of the variation for the GOM, MED, NSEA and WNAO populations 'all' data set, originates within populations and that 46.09% of the variation is attributed to the among population variation. Similar results (61.58% within and 38.42% among) were obtained for the GOM, MED, NSEA and WNAO 'restricted' data set comparison. All pairwise values, except those comparisons between the NSEA and WNAO, were highly significant ($P < 0.001$) for both F_{ST} and Φ_{ST}, and for both 'all' and 'restricted' comparisons (Table 5).

Table 2 Haplotype frequencies for four geographical putative populations

Haplotype	GOM	MED	NSEA	WNAO
A	0.026/*0.000*	0.000/*0.000*	0.444/*0.438*	0.429/*0.387*
B	0.150/*0.225*	0.000/*0.000*	0.111/*0.125*	0.095/*0.129*
C	0.072/*0.100*	1.000/*1.000*	0.444/*0.438*	0.440/*0.419*
X	0.686/*0.575*	0.000/*0.000*	0.000/*0.000*	0.012/*0.000*
Y	0.065/*0.100*	0.000/*0.000*	0.000/*0.000*	0.000/*0.000*
N	0.000/*0.000*	0.000/*0.000*	0.000/*0.000*	0.012/*0.032*
BB	0.000/*0.000*	0.000/*0.000*	0.000/*0.000*	0.012/*0.032*
Total	153/*40*	38/*20*	18/*16*	84/*31*

The left number indicates the 'all' data set and the right italicized number indicates the 'restricted' subset.

Microsatellite results

Sixteen microsatellite loci were compared among the GOM, MED and NSEA populations, and 13 loci were compared between WNAO and all other putative populations (see Materials and methods). Tests for linkage disequilibrium indicated independence among loci. Details of diversity and tests for deviation from HWE together with null allele estimates are shown in Appendix S1. Mean observed and expected levels of heterozygosity, over all 16 loci, are shown in Table 6. Most loci were within HWE or deviated only in one putative population, although a greater effect was seen for EV37 and D08. The analyses were run with and without these loci and in each case showed similar results and *P*-values (Table 7).

The results for F_{ST} and Rho_{ST} comparisons (Table 7) indicate that only the MED population was differentiated at microsatellite DNA loci. Results obtained from the model-based clustering method in STRUCTURE (Fig. 2) were consistent, indicating that the number of populations (*K*) equals two (average estimated ln probability

Table 3 Haplotype diversity (*h*), nucleotide diversity (π) and the mean number of pairwise differences within four geographical populations

Location	Data set	*n*	Haplotype diversity (*h*)	Nucleotide diversity (π)	Mean number of pairwise differences
GOM	All	153	0.500	0.002 ± 0.002	0.872 ± 0.616
	Restricted	40	0.614	0.003 ± 0.002	1.029 ± 0.702
MED	All	38	0.000	0.000 ± 0.000	0.000 ± 0.000
	Restricted	20	0.000	0.000 ± 0.000	0.000 ± 0.000
NSEA	All	18	0.628	0.003 ± 0.002	1.063 ± 0.737
	Restricted	16	0.642	0.003 ± 0.002	1.068 ± 0.744
WNAO	All	84	0.620	0.003 ± 0.002	1.091 ± 0.724
	Restricted	31	0.677	0.003 ± 0.002	1.158 ± 0.768
Overall	All	293	0.728	0.003 ± 0.002	1.282 ± 0.807
	Restricted	107	0.739	0.003 ± 0.002	1.284 ± 0.812

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Table 4 Exact test results for mtDNA population comparison among geographical populations

	Data set	n	GOM	MED	NSEA
GOM	All	153	—		
	Restricted	40			
MED	All	38	0.000 ± 0.000	—	
	Restricted	20	0.000 ± 0.000		
NSEA	All	18	0.000 ± 0.000	0.000 ± 0.000	—
	Restricted	16	0.000 ± 0.000	0.000 ± 0.000	
WNAO	All	84	0.000 ± 0.000	0.000 ± 0.000	1.000 ± 0.000
	Restricted	31	0.000 ± 0.000	0.000 ± 0.000	1.000 ± 0.000

P-values based on 100 000 steps in the Markov chain and 10 000 dememorization steps.
GOM, Gulf of Mexico; WNAO, Western North Atlantic Ocean; MED, Mediterranean Sea; NSEA, North Sea.

Table 5 mtDNA population comparison among geographical areas

	GOM	MED	NSEA	WNAO
GOM	—	0.460*	0.500*	0.482*
		*0.417**	*0.420**	*0.415**
MED	0.626*	—	0.600*	0.393*
	*0.586**		*0.510**	*0.412**
NSEA	0.425*	0.560*	—	−0.034
	*0.326**	*0.465**		*−0.047*
WNAO	0.409*	0.363*	−0.034	—
	*0.306**	*0.362**	*−0.046*	

F_{ST} values are presented in the lower left matrix and Φ_{ST} values are presented in the upper right matrix. '*Restricted*' data set values are in italics and provided below the 'all' data set values. Statistically significant P-values based on 10 000 permutations of the data and after Bonferroni corrections are marked with an asterisk (*P < 0.001).
GOM, Gulf of Mexico; WNAO, Western North Atlantic Ocean; MED, Mediterranean Sea; NSEA, North Sea.

for K = 1: −9902; K = 2: −9814; K = 3: −9932), with the MED sample differentiated from the other putative populations, which were not differentiated from each other. The possible influence of the geographically separate sample of 15 samples from the Caribbean in the putative WNAO population was tested by re-running comparisons without these samples (for both mtDNA and microsatellite DNA loci), and no difference was found (data not shown). AMOVA analyses based on the microsatellite DNA data were run including social groups within populations as a subdivision. The *restricted* data set confirmed that most variation was among individuals (99.71%). The unrestricted data set confirmed that our selection of nonkin to produce the *restricted* data set had removed kin structure at the social group level. For the unrestricted data, 93.18% of the variation was among individuals, while 5.01% was among social groups and 1.79% was among populations (all highly significant; P < 0.0001). All putative populations showed some indication of a population bottleneck based on excess heterozygotes (Table 8), but only for the IAM, and all showed the normal L-shaped mode-shift distribution.

Sex-biased dispersal

The results for population structuring were very different for mtDNA compared with nuclear DNA, suggesting sex-biased genetic dispersal. This was tested using analyses implemented in the program FSTAT (Goudet 2001; Table 9). The mean assignment test and H_S were each significant (P < 0.025) and provided values consistent with males being the more dispersing sex.

Discussion

A key objective in molecular ecology and conservation genetics is to discover patterns that suggest predictive trends. It will not be possible to assess the population structure of every threatened or endangered species, but hopefully we can learn enough about the essential life history and environmental characteristics that determine structure to make useful predictions by inference. Genetic dispersal determines continuity among populations, and it is reasonable to at first assess dispersal potential on the basis of the organism's life history, movement characteristics and physical potential for dispersal. For example, some fish species allow inference about the expected degree of panmixia because of the nature and abundance of their larval stages, although many competing factors can influence structure (e.g. Knutsen *et al*. 2007; Florin & Hoglund 2008). With marine mammals, there is a clear distinction between species tied to terrestrial breeding environments (e.g. the pinnipeds) and those that are fully aquatic (e.g. the cetaceans; see Hoelzel 2008). However, essentially all cetaceans have the physical potential for long-range dispersal, even though many show relatively fine-scale

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Table 6 Mean observed and expected heterozygosity levels for each population's respective data set

	Gulf of Mexico		Mediterranean Sea		North Sea		Western NAO*	
Heterozygosity	All	Restricted	All	Restricted	All	Restricted	All	Restricted
H_O	0.742 ± 0.162	0.750 ± 0.186	0.657 ± 0.205	0.685 ± 0.217	0.742 ± 0.158	0.741 ± 0.155	0.758 ± 0.143	0.770 ± 0.142
H_E	0.752 ± 0.133	0.759 ± 0.143	0.690 ± 0.191	0.711 ± 0.182	0.762 ± 0.142	0.766 ± 0.145	0.778 ± 0.130	0.791 ± 0.128

NAO, North Atlantic Ocean.
*Observed and expected values based on 13 loci.

Table 7 Pairwise comparisons using F_{ST} and Rho_{ST}

	GOM	MED	NSEA	WNAO
GOM	—	0.025*** (0.025)	0.004 (0.003)	0.000 (0.001)
		*0.057*** (0.025)*	*0.016 (0.005)*	*−0.007 (−0.001)*
MED	0.037*** (0.022)	—	0.022* (0.017)	0.038*** (0.032)
	*0.030*** (0.020)*		*0.034 (0.006)*	*0.063** (0.031)*
NSEA	0.000 (−0.002)	0.034*** (0.022)	—	−0.005 (−0.005)
	−0.005 (−0.004)	*0.029*** (0.021)*		*0.027 (0.000)*
WNAO	0.003 (0.003)	0.025*** (0.025)	−0.003 (−0.002)	—
	0.002 (0.002)	*0.015** (0.015)*	*−0.004 (−0.004)*	

F_{ST} values are presented in the lower left matrix and Rho_{ST} values are presented in the upper right matrix. '*Restricted*' data set values are in italics and provided below the 'all' data set values. Comparisons against the WNAO sets are based on 13 loci. Statistically significant *P*-values after Bonferroni corrections are marked with an asterisk (*0.01 < P < 0.05; **0.001 < P < 0.01; ***P < 0.001). Results for runs omitting EV37 and D08 shown parenthetically (significance values remained the same).
GOM, Gulf of Mexico; WNAO, Western North Atlantic Ocean; MED, Mediterranean Sea; NSEA, North Sea.

geographical structure among populations (see review in Hoelzel 2002). Foraging and breeding resource are key drivers for the larger cetaceans, some of which are known to migrate great distances between breeding and feeding grounds (see Stevick *et al*. 2002). Sperm whales do not have this type of predicable migration route, but have the physical stature to travel comparable distances. As for all mammals, there is the expectation that males will focus on females as a resource, while females focus on suitable environments for breeding and parturition (Greenwood 1980), and the distribution of both sexes is influenced by the distribution of their prey. For sperm whales, the consequent pattern of female philopatry and male dispersal would have the potential to be represented on an oceanic scale.

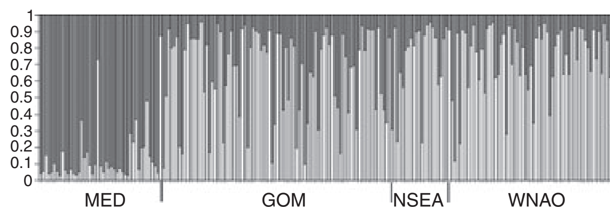

Fig. 2 Population structuring results based on the program STRUCTURE 2.2. Each whale is represented by a vertical bar with grey and white segments depending on the relative proportions of each putative population ancestry. Geographical regions where individuals were sampled are provided on the *x*-axis.

The re-sighting of individual sperm whales over periods of years, information on acoustic dialects and satellite-monitored tagging results all support the hypothesis that at least some female sperm whales exhibit site-fidelity to the GOM and MED coastal basins (Reeves & Notarbartolo di Sciara 2006; Jochens *et al.* 2008). Although little variation among putative populations had been detected in previous studies (see Introduction), our results revealed highly significant population structuring for the matrilineal mtDNA marker. Exact tests and comparisons for mtDNA F_{ST} and Φ_{ST} revealed strong differentiation between nearly all pairwise population comparisons for both the 'all' and 'restricted' data sets, even between the geographically proximate populations in the GOM and western North Atlantic. At the same time, the western North Atlantic sample was not differentiated from a sample at the other extreme end of the ocean basin, from coastal strandings in the NSEA (but note that these samples were from all male groups whose movement and behaviour may differ from the mixed sex groups).

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Table 8 Results from tests for population bottlenecks showing *P*-values for tests associated with excess heterozygotes (run in the program Bottleneck)

	IAM		TPM		SMM	
Location	Sign test	Wilcoxon	Sign test	Wilcoxon	Sign test	Wilcoxon
GOM	0.01	0.0002	0.33	0.096	0.01	0.97
WNAO	0.052	0.0012	0.33	0.34	0.039	0.98
NSEA	0.012	0.011	0.32	0.073	0.11	0.75
MED	0.0088	0.0012	0.37	0.034	0.55	0.39

GOM, Gulf of Mexico; WNAO, Western North Atlantic Ocean; MED, Mediterranean Sea; NSEA, North Sea; IAM, infinite allele model; TPM, two-phase mutation model; SMM, stepwise mutation model.

Table 9 Sex-biased dispersal results for males and females with respect to F_{IS}, F_{ST}, H_O, H_S, mean assignment and variance assignment

	n	F_{IS}	F_{ST}	H_O	H_S	Mean assignment	Variance assignment
Females	71	0.0009	0.045	0.733	0.733*	0.801*	13.659
Males	71	0.045	0.018	0.711	0.745*	−0.801*	14.451

*Statistically significant *P*-values after Bonferroni corrections ($P < 0.025$).

These results are striking, showing matriline differentiation over a small geographical scale, within compared to outside the GOM. Together, these data provide strong support for the interpretation that the MED and GOM basins are defining regional populations to which females are philopatric. Significant genetic differentiation between the North Atlantic and the MED has previously been reported for fin whales (*Balaenoptera physalus*), sperm whales and Cuvier's beaked whales (*Ziphius cavirostris*; Bérubé *et al.* 1998; Drouot *et al.* 2004; Dalebout *et al.* 2005) among other species. Restricted movement between both basins and the North Atlantic has been suggested for bluefin tuna (*Thunnus thynnus*; Boustany *et al.* 2008). Differentiation on either side of the Florida peninsula has been seen for a diversity of species (e.g. Dayan & Dillon 1995; Natoli *et al.* 2004).

Maintaining natal philopatry over time is not uncommon among female cetaceans or among female mammals in general. Although female sperm whales are capable of moving large distances (at least 4000 km; see Whitehead *et al.* 2008), factors such as foraging success, predator avoidance and social cohesion may all contribute towards the fact that average home ranges span only approximately 2200 km in any direction (Whitehead *et al.* 2008). At the same time, our data do not rule out female dispersal from natal groups or populations, and there are data to suggest that this does occur at some level (Best 1979; Kasuya & Miyashita 1988; Richard *et al.* 1996a; Whitehead 2003). For the GOM, the nutrient-rich outflow from the Mississippi river may be a factor influencing female philopatry.

The microsatellite DNA data, together with the comparative analysis of adult males and females, suggest that the pattern of movement may be quite different for males. There was significant differentiation between the MED whales and all other populations at these biparentally inherited loci, but at a low level. Regional differentiation at biparental markers had not been previously reported for this species over this geographical range. As discussed earlier, sex biases in mammalian dispersal may be related to advantages that occur for both males and females in competition for breeding resources or mates, or as a result of differential fecundity costs associated with dispersal for males and females (Greenwood 1980; Dobson 1982; Johnson 1986; Pusey 1987). Natal philopatry by both sexes is thought to be relatively rare (Greenwood 1980; Conner 2002), although there are a number of examples for odontocete cetaceans such as the killer whale (*Orcinus orca*; Hoelzel *et al.* 2007) and bottlenose dolphin (*Tursiops truncatus*; Natoli *et al.* 2005).

Male sperm whales range over huge distances, exploiting foraging opportunities at high latitudes (Teloni *et al.* 2008), expanding their range as they age and grow (Best 1979; Whitehead & Weilgart 2000). Discovery tags (deployed and recovered by whalers in the North Atlantic Ocean) have shown extreme cases of one male's longitudinal movement of 4300 km from the western to the eastern North Atlantic Ocean (Mitchell

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1975) and another male's latitudinal movement of 7400 km across the equator from North Africa to South Africa (Ivashin 1967). Our genetic data support an interpretation of long-range male-mediated genetic dispersal in this species, similar to that seen in the southern elephant seal (*Mirounga leonina*; Fabiani *et al.* 2003) and African elephant (*Loxodonta Africana*; Weilgart *et al.* 1996; Nyakaana & Arctander 1999). Dispersal by males from their natal site occurs for a variety of marine mammals (Baker *et al.* 1998; Escorza-Treviño & Dizon 2000; Moller & Beheregaray 2004), but evidence for dispersal at such a large geographical scale is relatively rare.

Data on population expansions and bottlenecks were somewhat equivocal, with some evidence for an historical expansion from the mismatch distributions, and a similar signal for a possible recent bottleneck in all four putative populations based on heterozygote excess (but only for the IAM). This is consistent with the idea of an historical event affecting all populations in the North Atlantic (as suggested earlier; Lyrholm *et al.* 1996), but less so with a scenario whereby the populations in the GOM and MED were established recently as founder populations. The latter effect could accentuate the difference between mtDNA and nuclear gene differentiation because of the influence of effective population size on coalescence.

In conclusion, while our study supports earlier results with respect to the low levels of variation found in this species (with no mtDNA variation at all found in the MED), it provides new evidence with respect to the structuring of populations within an ocean and among adjacent seas. The two major coastal basins on either side of the North Atlantic are seen to be home to philopatric populations of matrifocal social groups, from which males disperse, probably on an oceanic scale. With the ever-expanding oil and gas exploration and shipping industries encroaching on critical sperm whale habitat, we recommend that each population described here be classed as separate management units warranting protection measures aimed at promoting their long-term survival. We further suggest that this study implies transferable inference (by example) about the potential for female philopatry and male dispersal among populations on an oceanic scale for large, mobile marine species.

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All authors contribute to a better understanding of the population biology, behaviour, ecology and conservation needs of marine mammal species. Dan Engelhaupt undertook the laboratory work and analyses as a part of his PhD studies, supervised by Rus Hoelzel and with the assistance of technician Colin Nicholson. Work in the Molecular Ecology Group at Durham focuses on understanding the processes that govern the evolution of genetic diversity among and within natural populations in both marine and terrestrial environments.

Supporting information

Additional Supporting Information may be found in the online version of this article.

Appendix S1 Locus by population details on microsatellite DNA loci; k = number of alleles; n = sample size; null frequency is estimated using Microchecker.

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