RESEARCH ARTICLE

The influence of glacial epochs and habitat dependence on the diversity and phylogeography of a coastal dolphin species: *Lagenorhynchus albirostris*

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Abstract In this paper we use mitochondrial and micro-satellite DNA variation to investigate the mechanisms that underlie the evolution of population structure in a highly mobile marine mammal, the white-beaked dolphin. We found moderate genetic diversity (h) at mtDNA, but low nucleotide diversity (π) (0.7320 ± 0.0031 and 0.0056 ± 0.0004, respectively), consistent with expectations for a recent expansion. Analyses based on mismatch distributions further suggested a demographic expansion in the Norwegian-Barents Sea population and a spatial expansion in the British isles-North Sea population, implying distinct demographic histories. F_{ST} values showed clear differentiation among these two populations, but no difference was found between putative populations separated by the English Channel. Our data suggest a stepwise pattern of expansion, dependent on available coastal habitat. The conservation implications are a need to protect local populations isolated by an expanse of deep water, and in particular, a population along the British coasts and in the North Sea as separate from the North Norway-Barents Sea population. It is also evident that overall diversity was reduced, probably during the last glacial epoch.

Keywords Phylogeography · White-beaked dolphins · *Lagenorhynchus* · Population structure · North Atlantic · Spatial expansion · Demographic expansion

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Introduction

While logistical problems limit our ability to assess population structure in cetacean species (e.g. Milinkovitch et al. 2002), these data are never-the-less essential to the identification and conservation of diversity, and various factors have been shown to be relevant to the evolution of structure in these species. For example, historical processes, in particular the Pleistocene glaciations in the Northern Hemisphere, have been proposed as an important factor leading to genetic differentiation among marine species and populations, promoting speciation and influencing the distribution of lineages in coastal areas (e.g., Reeb and Avise 1990; Hewitt 2000; Hayano et al. 2004; Rosa et al. 2005; Adams et al. 2006; Haney et al. 2007). It has been hypothesized that during these climatic changes the distribution ranges of many marine species were restricted due to the creation of physical barriers (e.g., changes in sea level and sea temperatures) that reduced gene flow, even among proximate populations, and increased the effect of evolutionary forces such as genetic drift and selection (Avise et al. 1998; Hewitt 1996, 2000; Haney et al. 2007). After the end of the ice age, populations that were previously limited in small and specific areas probably expanded following a stepping stone model (dispersal occurring between neighbouring demes), or experienced a sudden demographic expansion, which could

explain the different patterns of genetic diversity found in contemporary northern populations (Slatkin and Hudson 1991; Rogers and Harpending 1992; Austerlitz et al. 1997; Ray et al. 2003; Excoffier 2004; Wegmann et al. 2006).

Another factor apparently shaping population structure in some dolphin species, especially those found primarily in coastal habitats is local habitat dependence. For example, Natoli et al. (2005) described population structure for the bottlenose dolphin (*Tursiops truncatus*) across a distribution from the Black Sea to Scotland, and found evidence for boundaries to gene flow at oceanographic borders (between the Black Sea, eastern Mediterranean, western Mediterranean, eastern North Atlantic and Scottish coastal waters). Various other marine species also show population genetic differentiation at these boundaries within the Mediterranean Sea (Bahri-Sfar et al. 2000; Guarniero et al. 2002; Perez-Losada et al. 2002). A common differential of habitat use in dolphin species, sometimes concomitant with high levels of genetic differentiation (e.g. Hoelzel et al. 1998a), is the use of nearshore versus offshore habitat (see review in Hoelzel 2009).

Here we provide a case study for the Atlantic white-beaked dolphin (*Lagenorhynchus albirostris* Gray 1846). This is the most northerly member of the genus *Lagenorhynchus* and is restricted to temperate and sub-polar seas in the North Atlantic (Leatherwood et al. 1976). White-beaked dolphins are considered a typical coastal species (Evans 1992; Mikkelsen and Lund 1994), and their spatial distribution and relative abundance along the Continental Shelf and coastal areas have been highly correlated with physical factors such as the presence of high marine productivity, sea temperatures (<12°C) and water depths (<120 m) as well as with ecological factors such as prey abundance, competition with other species and seasonal migration (Simard et al. 2006; MacLeod et al. 2007; Weir et al. 2007). Their distribution extends from southern New England, to Greenland, Iceland, Ireland, Scotland, Norway and south into the North Sea (Leatherwood et al. 1976; Evans 1992; Reeves et al. 1999).

Published estimates summarised by Hammond et al. (2008) indicate at least several thousand white-beaked dolphins in the western Atlantic, a few thousand around Iceland and up to a hundred thousand in Norwegian waters of the northeast Atlantic including the North Sea north of 65°N and particularly the Barents Sea. Hammond et al. (2002) using boat surveys estimated the population size of *L. albirostris* in the North Sea and Channel at 7856 individuals (CV = 0.30). Although these numbers sound fairly substantial for some regions, there are ongoing threats and local impact.

It is well known that bycatch in fisheries, over-fishing of prey, degradation or loss of coastal habitats and pollution, among others, are the principal causes leading to vulnerability or extinction of coastal species (Rosel et al. 1999; Cassens et al. 2005,). For *L. albirostris* in particular there has been a long tradition of small scale hunting in several countries including Faroe Island, Greenland, Iceland and Norway, mainly for food. Hunting may still continue in some areas, e.g. at the southwest coast of Greenland, in Labrador and the Faroe Islands (Jefferson et al. 1993; Reeves et al. 1999; Lien et al. 2001). Alling and Whitehead (1987) have claimed that approximately 366 individuals of *L. albirostris* were killed for subsistence in northern and southern Labrador each year. Moreover, individuals of this species are frequently taken in a variety of fishing gear throughout their range (IUCN 2007). Persistent impact could translate into a reduction of effective population size, and the resulting loss in diversity that could affect their capacity to respond to environmental changes (Castello 1996; Rosel et al. 1999; Pichler and Baker 2000; Cassens et al. 2005).

At the same time, little is known about the population structure of *L. albirostris* in the North Atlantic and North Sea. Suggestions about population stocks in this species have been mainly drawn from short-term, localized sightings surveys (e.g., Northridge et al. 1995; Hammond et al. 2002; Evans and Hammond 2004). Based on these surveys, Northridge et al. (1995, 1997) suggested a possible stock separation in the eastern North Atlantic, with individuals in the North Sea and around the British Isles being a distinct population from those found in the northwest of these regions. In contrast, Mikkelsen and Lund (1994), using Principal Component Analysis (PC) and Partial Least Square analysis (PLS) on the skull features of 179 specimens of *L. albirostris*, together with 20 non-metrical characters, found no significant differentiation among individuals from the eastern North Atlantic. However, these analyses showed non-overlapping distributions among samples from the western North Atlantic (United States coasts) and European animals (mainly for the North Sea and English channel), suggesting at least two sub-populations ($P < 0.2\%$).

In order to facilitate the conservation of *L. albirostris* populations, it is important to build on these existing data to provide a better understanding of the pattern of population genetic structure, and the mechanisms that lead to its evolution in this species. Here we evaluate two main hypotheses using data from the analysis of mtDNA and microsatellite DNA loci. First, as for a number of species in the North Atlantic (e.g., Wares and Cunningham 2001; Wares 2002; Carr and Marshall 2008; Domingues et al. 2008) we expect to find signals for refugial bottlenecks and subsequent expansions. Second, the habitat dependence of this species on coastal regions may affect both the pattern of expansion and the pattern of contemporary gene flow among populations, with coastal regions acting like habitat

islands where local populations could become isolated. In particular, we test the hypothesis that gene flow and spatial expansion will mostly follow contiguous or proximate regions along coastal habitats.

Materials and methods

Sampling collection and extraction

DNA from 116 white-beaked dolphins (*L. albirostris*), including tissue, bones and teeth, was extracted from samples collected in the eastern and western North Atlantic (Fig. 1). Bone and teeth samples were collected from the Museum of Natural History of Rotterdam, the Museum of Natural History in Leiden, and the Smithsonian Institute in the United States. Samples from living and stranded dolphins were obtained from stranding network collections in Scotland and England, and through the Institute of Marine Research, Norway. Four putative populations were represented—the western North Atlantic (WNA), UK waters (UK), The Netherlands, and Norwegian-Barents Sea (Norway). Total genomic DNA was extracted from tissue following the procedure recommended by Hoelzel and Green

(1998). The teeth or bones were ground using a pestle and mortar (autoclaved between extractions), and digested in 2 ml lysis solution for 48 h at 37°C (0.5 M EDTA, pH 8.0, 0.1 M Tris–HCl and 0.5% SDS). After digestion the DNA was extracted using a Qiagen PCR purification kit.

Mitochondrial control region (mtDNA) amplification and sequencing

Two fragments of the maternally inherited mtDNA control region were amplified under the following conditions: 20–50 ng of DNA, $10 \times$ PCR buffer, 1.5 mM $MgCl_2$, 50–100 ng of primers, 2.5 mM dNTP and 1 U of Taq polymerase (except for bone samples, where 2 U was used). Amplifications were conducted with the following cycle conditions: 94°C 2 min followed by 35 cycles of 94°C 30 s (46 cycles in teeth and bone samples), 54°C 30 s and 72°C 30 s. A 601 bp fragment was amplified using universal primers MTCRF (5′-TTC CCC GGT CTT GTA AAC C-3′) and MTCR-R (5′-ATT TTC AGT GTC TTG CTT T-3′) from Hoelzel and Green (1998). This 601 bp fragment did not amplify in bone and teeth samples; thus internal primers (flanking internal regions in the 601 bp fragment) were used to amplify a smaller fragment (323 bp) (AcuF:
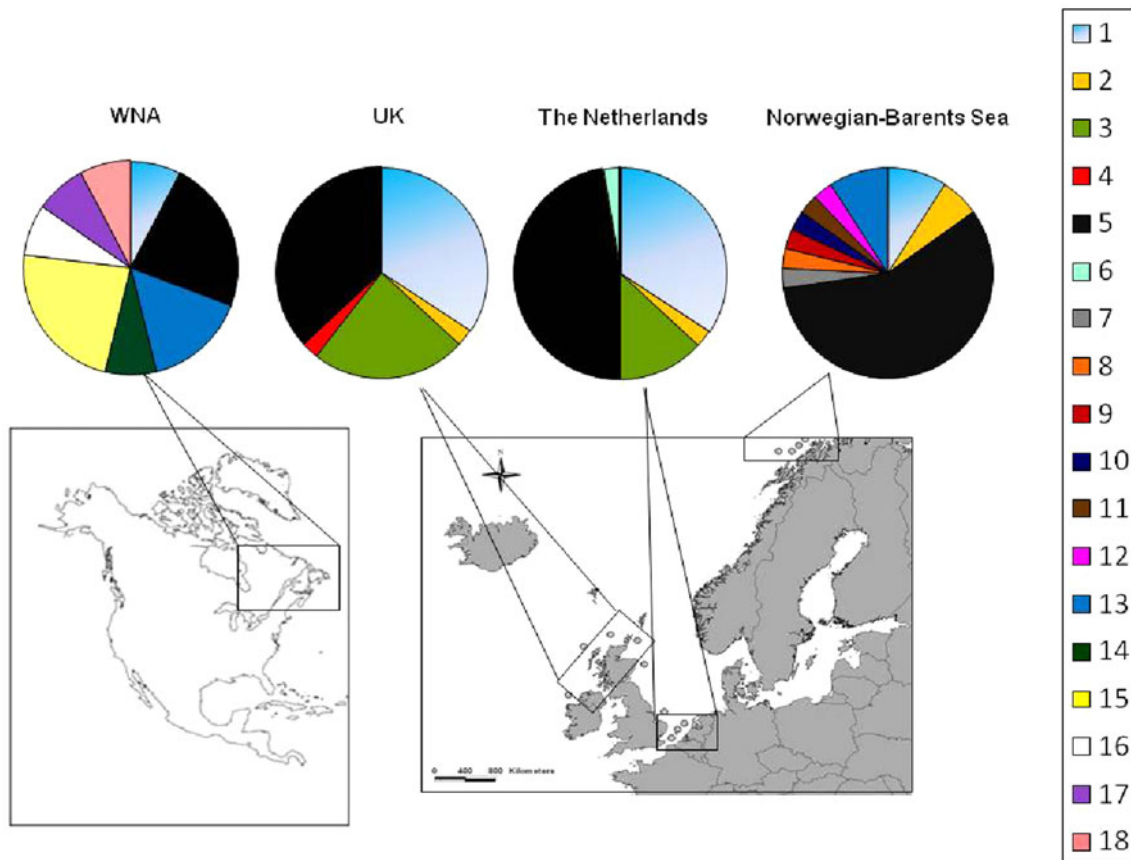
Fig. 1 Distribution of haplotypes for 323 bp mtDNA control regions (c.f. Fig. 3 for haplotype frequencies; color version available online)

5′-TGT ACA TGC TAT GTA TTA T-3′ AcuR: GCT TTA ACT TAT CGT ATG G-3′). After amplification the samples were purified using Qiagen columns (Qiagen, Inc.) and directly sequenced in an ABI 377 automated sequencer. The sequences were aligned using the Clustal X programme (1.83) from Thompson et al. (1997) and edited using the programme Chromas Pro (www.technelysium. co.au). After sequencing, samples were divided into three regions in the eastern North Atlantic (ENA): The Netherlands ($N = 38$), Norway ($N = 33$) and the United Kingdom (UK; $N = 38$), and one region in the western North Atlantic (WNA; $N = 13$). The WNA sample includes seven from Canada collected for this study and six from Gen-Bank (accession No: EF092928, EF092930, EF092929, AJ554061, EF092931 and EF092932).

The extent of genetic variation in the control region was assessed by examining both haplotype (h) and nucleotide diversity (π), using Arlequin v 3.11 (Excoffier et al. 2007) and DNAsp v 4.0 (Rozas et al. 2003). The variance components of gene frequencies were partitioned between geographic regions (groups), and differentiation was quantified using the fixation index, F_{ST} (Wright 1951; Excoffier et al. 1992). The statistical significance of the variance components and fixation statistics were tested using a non-parametric permutation approach with 10,000 permutations. The WNA sample size was too small to be usefully included in these analyses.

Phylogenetic relationships among haplotypes were examined by generating a neighbour-joining tree for the complete set of mtDNA haplotypes using the Tamura and Nei substitution model (chosen to control for rate variation across the sequence; Tamura and Nei 1993). These analyses were conducted using MEGA version 4.0 (Tamura et al. 2007) and PAUP* v. 4.0b10 (Swofford 2002). In addition, a median-joining network tree was built to infer the ancestral relationships among haplotypes using the programme Network, version 4.5.0.0 (Bandelt et al. 1999).

Demographic history was assessed using the mismatch distribution (Rogers and Harpending 1992). Fit to the model was evaluated using the sum of square deviations (SSD) between the observed and the expected mismatch, and the raggedness index (r) of the observed distribution (Harpending 1994; Schneider and Excoffier 1999). Given that coalescent methods are generally more robust for small sample sizes, the WNA sample was included in these analyses. Expectations based on neutrality were assessed using Fu's Fs (Fu 1997) and Tajima's D (Tajima 1989) in the program Arlequin v 3.11 (Excoffier et al. 2007). The significance of Tajima's D was determined by generating 1,000 random samples under the assumption of selective neutrality with a coalescent simulation algorithm (Hudson 1990).

A model of spatial expansion was also evaluated. The programme Arlequin 3.11 (Excoffier et al. 2007) was used to derive the expected mismatch distribution under the continent-island model (equivalent to an infinite island model; see Excoffier 2004), which assumes that genes were sampled from a single deme, and belong to a population subdivided into an infinite number of demes of size N that exchange m migrants with other demes. Three parameters of spatial expansion were estimated: τ, $\theta = \theta_0 = \theta_1$ assuming $N = No$ and $M = Nm$ using a least-square method. The fit to the model was tested by coalescent simulations, assuming an instantaneous expansion under the continent-island model as describe by Excoffier (2004).

The coalescence time of expansion in years (t) was calculated using the relationship $\tau = 2vt$, where τ represents the mode of the mismatch distribution (in units of evolutionary time) and v is the mutation rate for the sequence used. The v value was calculated as suggested by Rogers and Harpending (1992), using the formula $v = \mu k$, where μ is the mutation rate per nucleotide per year and k is the number of nucleotides evaluated. Demographic parameters were estimated using two different evolutionary rates: (i) The estimate by Harlin et al. (2003) ($\mu = 7.0 \times 10^{-8}$) based on comparisons between *Phocoena phocoena* and *L. obscurus* (ii) A recent estimate of mutation rate for the control region of $\mu = 5 \times 10^{-7}$ by Ho et al. (2007) based on data incorporated from ancient DNA.

A Bayesian sampling coalescent approach implemented in the Mdiv program (Nielsen and Wakeley 2001) was used to evaluate whether or not the observed genetic pattern in the populations of *L. albirostris* studied fit an isolation-with-migration model under the finite site mutation model (HKY) (Hasegawa et al. 1985). The Mdiv programme was run twice using 5×10^6 and 10×10^6 chains and a burn-in of 10% (500000 and 1000000, respectively) as recommended by the authors.

Microsatellite loci

Six novel microsatellite DNA loci were developed for this study using the protocol developed by Carleton et al. (2002). A blue-white screening was used to select positive colonies, and the insert size was tested by PCR using two universal primers (M13 reverse and forward) and one microsatellite-specific primer (5′-TGT GGC GGC CGC (TG)₈-3′). The positive colonies were grown in overnight cultures of 6–8 ml at 37°C; the culture was miniprepped using a GeneElute™ Plasmid Mini-prep Kit (Sigma). Each clone was cut with ECORI and 26 positive clones with fragment above 400 bp were sequenced in one direction in an ABI 377. Six clones showed associated microsatellites of 15, 20, 54, 68, 80 and 128 di-nucleotide repeats (CA) and were used to design microsatellite-specific primers using the Oligo programme.

Six sets of primers were designed, but only four microsatellites were successfully standardized (*Lalb6a,*

Lalb3a, Lalb15a and Lalb32a) and an additional 11 cetacean-specific loci were included as follows: D22 (Shinohara et al. 1997), EV37 and EV94 (Valsecchi and Amos 1996), FCB4 (Buchanan et al. 1996) GT136 (Andersen et al. 2001), KWM2a (Hoelzel et al. 1998b), Textvet7 (Rooney et al. 1999), Lobs Di9, LobsDi19, LobsDi24, LobsDi47 (Cassens et al. 2005). The PCR reactions were performed in the presence of 20–50 ng of DNA for tissue samples (10 µl of the DNA solution for teeth samples) for a final volume of 20 µl. The reaction mix contained 200 nM of each primer (the forward primer was labelled using fluorescence to allow detection by the program sequencer), 0.5–0.75 mM $MgCl_2$, 0.1–0.36 mM dNTPs and 0.2 U Taq polymerase (Bioline).

The PCR conditions for primers D22, GT136, FCB4 and Kwm2a were: Denaturation at 95°C for 5 min, 35 cycles at 94°C for 45 s, 1 min 30 s at locus-specific annealing temperature, extension at 72°C for 1 min 30 s. PCR conditions for Textvet 7: Denaturation at 95°C for 5 min 35 cycles at 94°C for 40 s, 1 min 30 s at locus-specific annealing temperature and 1 min 40 s at 72°C. Loci Di19, Di 24, Di 9 and Di 47 were amplified using the PCR conditions described by Cassens et al. (2005) and for PCR conditions for Ev37 and Ev94 see Valsecchi and Amos (1996).

The PCR conditions for the specific primers for *L. albirostris* (*Lalb6a, Lalb3a, Lalb15a and Lalb32a*) were as follows: Primer denaturation at 95°C for 5 min, 35 cycles at 94°C for 45 s, 1 min 30 s at locus-specific annealing temperature, and extension at 72°C for 1 min 30 s followed by 5 min final extension. Given the small number of samples available for the WNA, differentiation at microsatellite loci was evaluated only among the eastern North Atlantic populations (Norway, UK and The Netherlands).

To identify and correct genotyping errors (i.e. to check evidence for scoring error due to stuttering, large allele dropout or evidence for null alleles), the program Microchecker (Van Oosterhout et al. 2004) was used. Microsatellite variation was examined by estimating the number of alleles per locus, gene diversity and allelic richness using the programme Fstat vers. 2.9.3 (Goudet 2001). Regional differences in frequencies and deviation from the Hardy–Weinberg equilibrium were tested using the GENEPOP 1.2 programme (Raymond and Rousset 1995) and Arlequin v 3.11 (Excoffier et al. 2007). To test the null hypothesis of independence between genotypes, evidence for linkage disequilibrium was tested using Fstat vers. 2.9.3 (Goudet 2001).

The heterozygote deficiency test and the heterozygote excess test (Rousset and Raymond 1995) were calculated and subjected to sequential Bonferroni correction (Rice 1989). A Markov chain estimate of Fisher's exact test was also used in order to test the null hypothesis that allelic distribution was identical across populations (Guo and Thompson 1992). Population differentiation was assessed between the Norwegian and UK populations comparing 14 microsatellites (excluding *Lalb3a,* see results) and between Norwegian and The Netherlands and UK and The Netherlands using five microsatellites performing the fixation index (F_{ST}) approach of Weir and Cockerham (1984) and R_{ST} (Slatkin 1995).

To test the hypothesis that the populations are in mutation-drift equilibrium, the Wilcoxon signed-rank test in the Bottleneck programme (Cornuet and Luikart 1996) was used. This programme evaluates the differences between observed and expected heterozygosities across all loci in a population sample. In a population that underwent a bottleneck, a transient excess of heterozygosity is expected; thus a higher-than-expected observed heterozygosity would be found (Cornuet and Luikart 1996). The analyses were performed using 12 microsatellite loci, excluding loci that presented deviation of HW equilibrium (Ev94 and Ev37). The three mutation models proposed for microsatellite data and implemented by the program BOTTLENECK were evaluated using 1000 interactions: the Infinite Allele Model (IAM, Kimura and Crow 1964) the Stepwise Mutation Model (SMM, Kimura and Ohta 1978) and the Two Phase Model (TPM, Di Rienzo et al. 1994). The TPM model was run using a proportion of SMM equal to 70%. The reduction in population size was also tested with the statistic *M*, proposed by Garza and Williamson (2001) and implemented in the programme Arlequin.

Results

Genetic variation

One hundred and 22 samples (including six samples from the NCBI GenBank data base-see above) were analyzed using two fragments of the mtDNA control region. Sequences from tooth samples from The Netherlands and the WNA were restricted in length (323 bp) while the UK and Norway populations could be compared using both the 323 bp and 601 bp fragments. Table 1 shows the details of haplotypic and nucleotide diversity for the various samples. Both measures were relatively low compared to other cetacean species (see review in Hoelzel et al. 2002), with the effect more pronounced for nucleotide diversity. The overall genetic diversity for the 601 bp fragment (UK plus Norway) was relatively high (0.868 ± 0.003) although these values dropped when the smaller fragment was analyzed (0.722 ± 0.043). The distribution of haplotypes among putative populations is shown in Figs. 1 and 2. The UK and Norway populations shared four haplotypes, with 12 private haplotypes in Norway (Fig. 2). Eighteen haplotypes were found among western and eastern North

Table 1 Genetic diversity at the mtDNA control region in *L. albirostris*

Population	N	Number of haplotypes	Number of polymorphic sites	Haplotype diversity (H)	Nucleotide diversity (π)
323 bp fragment					
UK	38	5	5	0.7084 ± 0.0321	0.0041 ± 0.0031
Norway	33	10	11	0.6630 ± 0.0900	0.0055 ± 0.0036
The Netherlands	38	5	5	0.6572 ± 0.0476	0.0043 ± 0.0029
WNA	13	8	12	0.9103 ± 0.0559	0.0096 ± 0.0060
601 bp fragment					
UK	29	7	8	0.8128 ± 0.0385	0.0044 ± 0.0027
Norway	33	19	19	0.8788 ± 0.0480	0.0062 ± 0.0036
Overall					
ENA 323 bp	109	13	13	0.6970 ± 0.0320	0.0050 ± 0.0003
ENA (UK–Norway) 601 bp	62	19	21	0.8680 ± 0.0033	0.0059 ± 0.0004
WNA and ENA	122	18	21	0.7320 ± 0.0031	0.0056 ± 0.0004

	22	73	88	95	99	100	136	156	166	218	245	248	329	391	392	457	458	539	542	592	599	NORWAY	UK
Hap 1	T	A	G	T	C	A	T	G	T	C	T	A	A	T	C	C	C	A	C	G	C	1	
Hap 2	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	11	9
Hap 3	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	2	
Hap 4	.	.	C	.	.	.	.	.	.	.	C	.	G	.	.	.	.	.	.	.	.	1	
Hap 5	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	1	
Hap 6	.	.	C	.	.	.	.	.	.	.	.	G	.	.	.	T	.	.	.	.	.	1	
Hap 7	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	3	
Hap 8	.	.	C	.	.	.	.	.	.	.	.	G	.	.	.	T	.	.	.	.	.	2	
Hap 9	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	1	3
Hap 10	.	.	C	.	.	.	.	C	C	T	.	.	.	.	.	.	.	.	.	.	.	1	
Hap 11	.	.	C	.	.	.	.	C	C	T	.	.	G	.	.	.	.	.	.	.	.	1	
Hap 12	.	.	C	.	.	.	.	C	.	T	.	.	.	.	.	T	.	G	T	.	.	2	1
Hap 13	.	.	C	.	.	.	.	C	.	T	C	.	G	.	.	T	.	G	T	.	.	1	
Hap 14	.	T	C	.	.	.	.	C	.	T	.	.	.	.	.	T	.	.	T	.	.	3	
Hap 15	.	.	C	.	.	.	.	C	.	T	.	.	.	.	.	T	.	G	.	.	.	1	6
Hap 16	.	.	C	.	.	.	.	C	.	T	.	.	.	.	A	.	T	G	.	.	.	0	2
Hap 17	.	.	C	.	.	.	.	C	.	T	.	.	.	.	.	T	.	G	.	A	.	0	1
Hap 18	.	.	C	.	.	C	G	C	.	T	.	.	.	.	.	T	.	G	.	T	T	1	
Hap 19	C	.	C	.	.	.	.	C	.	T	.	.	.	.	.	T	.	G	.	.	.	0	7

Fig. 2 Polymorphic sites and haplotypes in UK and Norway populations (601 bp mtDNA control region)

Atlantic populations, defined by 21 polymorphic sites, using 323 bp. Only two haplotypes were shared between all four putative populations (Fig. 1; accession numbers: HM047744–HM047761).

No linkage disequilibrium was found among micro-satellite locus pairs. Two loci, EV37 and EV94, showed evidence for null alleles and one locus showed evidence for errors due to stuttering, based on analysis using the program Microchecker (Van Oosterhout et al. 2004). The locus with the stutter errors (Lalb3a) was deleted from further analyses. After Bonferroni correction, loci Ev37 and Ev94 showed deviation from HW in the UK and Norwegian populations and Di24 in The Netherlands population. Excluding these did not change the results (data not shown), and so they were retained for all analyses except the tests using the program BOTTLE-NECK. Microsatellites diversity statistics are shown in Table 2.

Table 2 Genetic diversity at microsatellite loci in *L. albirostris*

Locus	Population	N	K	R	Ho	He	Fis	Allelic range	G–W statistics
Di9	UK	23	4	4.0000	0.4783	0.4918	0.0280	8	0.4444
	Norway	37	4	3.5980	0.2703	0.2477	−0.0930	10	0.3636
	The Netherlands	25	4	4.0000	0.5385	0.5784	0.0700	15	0.3125
Di47	UK	26	3	2.9890	0.2308	0.2745	0.1620	6	0.4286
	Norway	42	6	5.5870	0.5238	0.5562	0.0590	16	0.3529
	The Netherlands	21	6	6.0000	0.5717	0.5700	0.3300	10	0.3636
Di24	UK	26	7	6.8720	0.6539	0.7768	0.1610	20	0.3333
	Norway	43	8	7.3950	0.7674	0.8142	0.0580	16	0.4706
	The Netherlands	23	8	7.9170	0.7500	0.8174**	0.0840	14	0.4667
Di19	UK	26	3	3.0000	0.6923	0.5935	−0.1700	13	0.2143
	Norway	44	5	4.4460	0.4318	0.5180	0.1680	25	0.1923
Texvet7	UK	26	5	4.9990	1.0000	0.7436	−0.3540*	14	0.3333
	Norway	44	4	3.8950	0.7727	0.6685	−0.1580	10	0.3636
	The Netherlands	26	5	4.8730	0.5769	0.7006	0.1790	20	0.2857
Gt136	UK	26	4	3.9990	0.6923	0.6267	−0.1070	18	0.2105
	Norway	44	5	4.5490	0.6818	0.6136	−0.1130	10	0.4545
D22	UK	23	7	7.0000	0.7391	0.6976	−0.061*	20	0.3333
	Norway	42	5	3.6430	0.6905	0.5204	−0.3320	10	0.4545
	The Netherlands	25	5	4.9950	0.8000	0.6996	−0.1470	8	0.5556
Lalb6a	UK	26	8	7.7570	0.6154	0.7481	0.1800	32	0.2424
	Norway	44	10	8.5240	0.6818	0.7537	0.0960	22	0.4348
Lalb32a	UK	26	7	6.7570	0.7308	0.6659	−0.1000	33	0.2059
	Norway	43	8	6.5520	0.5349	0.6227	0.1420	33	0.2353
FCB4	UK	25	4	3.9200	0.7200	0.5845	−0.2380	6	0.5714
	Norway	41	6	5.3570	0.6829	0.6555	−0.0420	12	0.4615
EV37	UK	24	4	3.9990	0.3750	0.6277**	0.4080	28	0.1379
	Norway	44	10	8.9860	0.6591	0.6904	0.0460	32	0.3030
KWM2a	UK	26	6	5.7580	0.6154	0.6772	0.0930	14	0.4000
	Norway	41	4	3.5610	0.6098	0.5959	−0.0240	14	0.2667
EV94	UK	24	6	5.8740	0.2500	0.4220**	0.4130	14	0.4000
	Norway	43	7	6.1350	0.4186	0.4208	0.0050	14	0.4667
Lalb15a	UK	24	10	9.8320	0.7917	0.7642	−0.0370	22	0.4348
	Norway	31	6	5.8090	1.0000	0.6563	−0.537*	12	0.4615
Overall microsatellites (14)	UK		5.57	5.4830	0.6132	0.6210	0.0130	17.71	0.3350
	Norway		6.29	5.5740	0.6233	0.5453	−0.0480	16.85	0.3772
Overall microsatellites (5)	The Netherlands		5.40	5.2600	0.6374	0.6732	0.2180	13.4	0.3968

Fis: Degree of random mating as in Weir and Cockerham (1984). N: number of samples; K: number of alleles; R: Allelic Richness; G–W: Garza–Williamson statistics; Ho: Observed heterozygosity; He: Expected Heterozygosity. Samples with deviation from H–W equilibrium are denoted with two asterisks (**) and significant values of Fis are shown with one asterisk (*)

Differentiation among populations and phylogenetic relationships

F_{ST} and φ_{ST} values for the mtDNA control region and microsatellite DNA loci are shown in Table 3. The UK and The Netherlands were not differentiated, but Norway was from both of the other two. Although the sample size for the WNA population was small, the pattern of haplotype frequencies was clearly distinct (Fig. 1).

Both the neighbour joining phylogenies (using either length sequence) and median-joining network show a certain degree of association between haplotypes from each region, especially those from Norway, but with relatively low bootstrap support in the Neighbour joining tree (Fig. 3). One haplotype at the centre of a star phylogeny structure (haplotype 5) is relatively abundant in all putative populations, while another (haplotype 1) is most common in the UK and Netherlands. The strongest expansion signal

Table 3 φ_{ST} and F_{ST} values for *L. albirostris* using the mtDNA control region (below diagonal) and R_{ST} and F_{ST} values based on microsatellite DNA loci (above diagonal)

Region	UK	The Netherlands	Norway
UK		0.0106	**0.0227 (14 loci)**
		0.0041	**0.0185**
The Netherlands	−0.00149		**0.0139 (5 loci)**
	−0.0092		**0.0285**
Norway	*0.1360*	**0.0605**	
	0.0918	**0.0498**	

Bottom value in each cell is the F_{ST} value. Values in italics were calculated using 601 bp, values in bold are significant at the 0.05 level (after Bonferroni correction)

based on the phylogenies is seen in the Norwegian sample where haplotype 5 is found at a frequency of 58%, and no other haplotype exceeds 9%. In most cases the mismatch distributions from individual population samples were not clearly unimodal, (Fig. 4); however, the values of the variance (SSD) and the raggedness index (*r*) were small and non-significant in all populations (for both models), suggesting that the distributions did not differ significantly from those expected under a model of sudden demographic or spatial expansion (see Roger and Harpending 1992; Schneider and Excoffier 1999; Table 4) Tajima's D and the Fu's Fs statistics (based on the longer sequences) were all positive and non-significant (Tajima's D: UK: 0.85, $P = 0.82$; The Netherlands: 0.38, $P = 0.69$; Fu's Fs: UK: 0.33, $P = 0.60$; The Netherlands: 0.48, $P = 0.64$), except for the Norwegian population (Fu's Fs = −5.7633, $P = 0.0095$; Tajima's D: 0.85, $P = 0.82$).

Given that there is no evidence to reject the hypothesis of demographic expansion or spatial expansion in *L. albirostris*, τ values were used to calculate expansion times using two different evolutionary rates (see above; Table 4). Note that in every case the length of the sequence affected the estimate of tau, and therefore on the assumption that the longer sequence provided better resolution, further interpretation was based on expansion times derived from the longer sequence.

Three population pairs were compared using the isolation-with-migration model in the program MDIV (UK versus Norway, WNA versus Norway and WNA versus UK). All parameters had the same distribution when using different chains and burn-in steps (data not shown). The isolation with migration analysis suggested that the ancestral migration between Norway and WNA populations (~ 4 females every five generations) was lower than ancestral migration between UK versus WNA (~ 2 females per generation), however given the small sample sizes for the WNA population and the absence of samples from contiguous regions, this results should be interpreted with

caution. Splitting times could not be resolved for any of the population pairs analyzed. However, the time to the most recent common ancestor (TMRCA) was estimated (using MDIV), and the following values were obtained using $\mu_1 = 5 \times 10^{-7}$ (Ho et al. 2007) and $\mu_2 = 7 \times 10^{-8}$ (Harlin et al. 2003) (UK–WNA: TMRCA$\mu_1 = 10,433$ TMRCA$\mu_2 = 74,524$; Norway–WNA: TMRCA$\mu_1 = 14,012$ TMRCA$\mu_2 = 100,088$; UK–Norway: TMRCA$\mu_1 = 6,140$ TMRCA$\mu_2 = 43,855$).

In order to address whether the populations of *L. albirostris* have experienced further reduction in population sizes due to a recent bottleneck, the program BOTTLENECK and the M value proposed by Garza and Williamson (2001) were analyzed. For the British Isles-North Sea population the Wilcoxon test gave a significant results for excess gene diversity under the assumption of the IAM model ($P = 0.03418$) and under the SMM model ($P = 0.006$), but results were not significant under the TPM model ($P = 0.677$). For the Norwegian population the test gave significant result only under the SMM model ($P = 0.001$). Values from Garza's M were low (≤ 0.4) and consistent with expectations for populations that have undergone a bottleneck.

Discussion

Genetic diversity and demographic expansions

Diversity at the nuclear level in *L. albirostris* was moderate, and similar to values reported for other delphinids populations (e.g., Buchanan et al. 1996; Hayano et al. 2004). The mitochondrial haplotypic diversity was also within the range described for other cetacean species (e.g., Pichler and Baker 2000; Harlin et al. 2003; Cassens et al. 2003; Hayano et al. 2004; Natoli et al. 2006; Querouil et al. 2007). In contrast, the nucleotide diversity was very low (ranging from 0.0043 in The Netherlands to 0.0096 in the WNA population) and similar to values reported for cetacean populations with historically small population sizes or that have been strongly affected by human activities (e.g. Bérubé et al. 1998; Natoli et al. 2006). This pattern is expected when diversity is lost (for example through a bottleneck event), and then regained after a population expansion.

Given this species' limited distribution, an impact on population size during Pleistocene glaciations, as proposed for other temperate species, is a credible scenario (see Wares 2002; Hewitt 2000, 2004). This hypothesis was supported by the mismatch distribution analyses to some extent, and by the *M*-ratio data suggesting an older bottleneck. However, most of the mismatch distributions were not strongly unimodal, limiting the strength of this interpretation (a non-significant deviation from the model
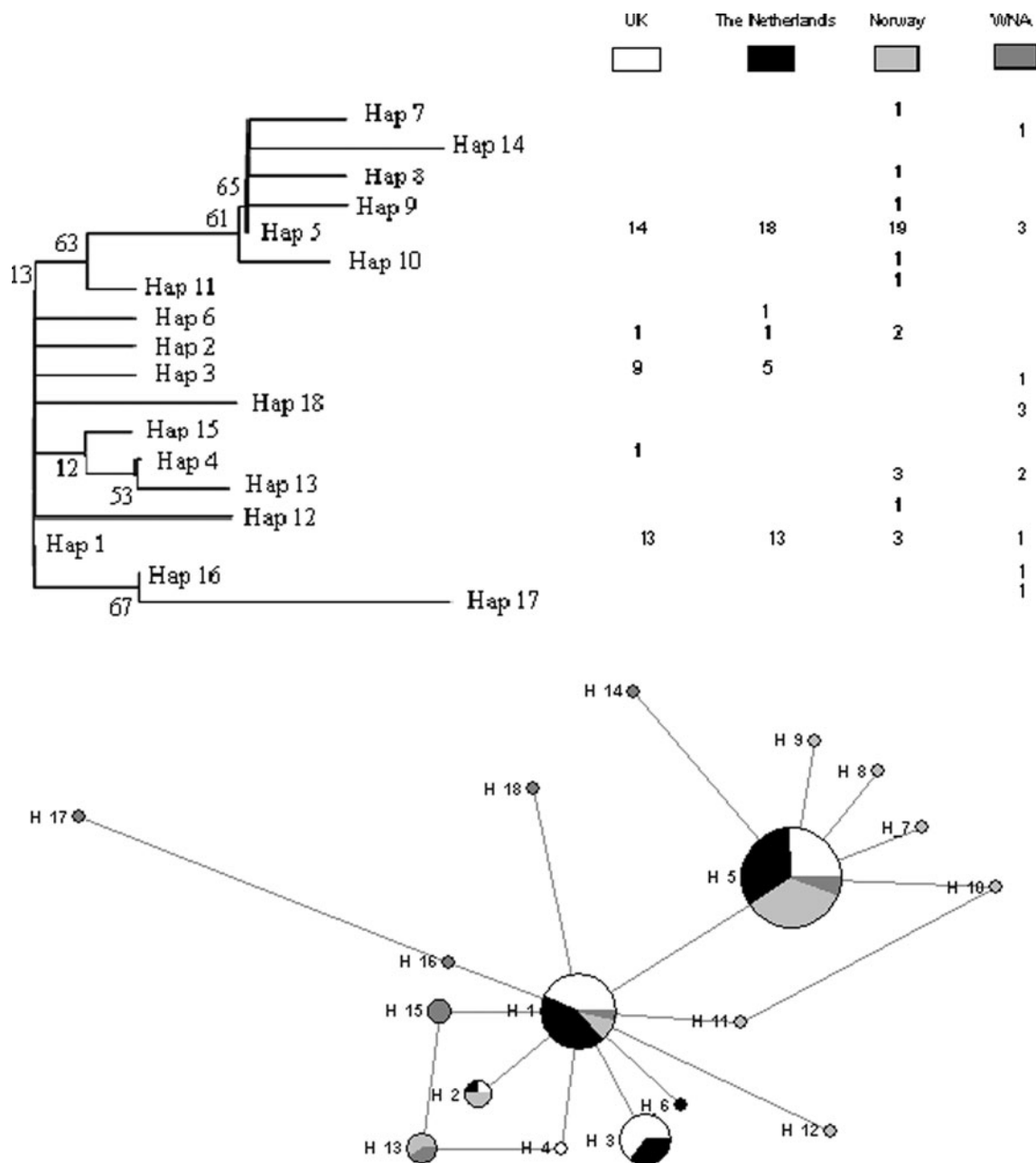
Fig. 3 Neighbour joining and Network tree showing the relationships among haplotypes using 323 bp. Shades in the NJ tree represent private haplotypes for each region. Line lengths in the network tree are proportional to the number of mutations among haplotypes

distribution does not necessarily mean that the mismatch distribution is unimodal). A more recent expansion in Norway is supported by the phylogenetic reconstructions, the relatively clear unimodal shape of the mismatch distribution, and the negative and significant Fu's Fs statistic. Some results from the program Bottleneck also suggested a population contraction and expansion in both the UK and Norway (significant evidence of gene diversity excess based on the relatively conservative SMM for both populations).

The fact that the same haplotypes are common among distant populations and the star-like shape of the network around two dominant haplotypes are consistent with the interpretation of an earlier expansion (see Fig. 3), predating population subdivision in this species. Only the fastest mutation rate estimates would suggest a post-glacial expansion (see Table 4), but these fast rates for this locus have provided credible time points for demographic expansions in a variety of species, such as bison responding first to changing climate, and then to the arrival of human
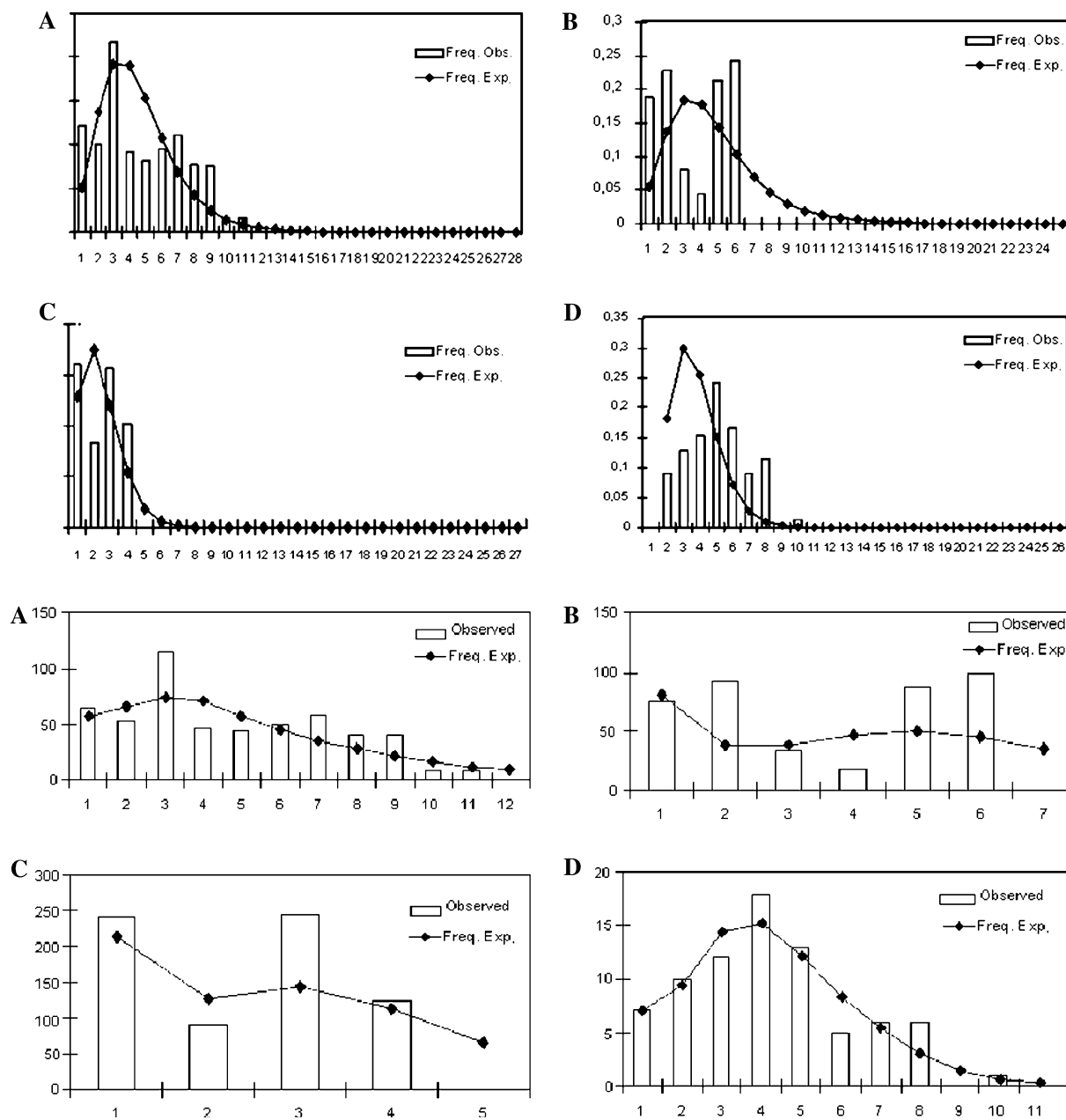
Fig. 4 Mismatch distribution under a model of demographic expansion (*upper graph*) and under a model of spatial expansion (*lower graph*). The x axis shows the number of pairwise differences, the y axis shows the frequency of the pairwise comparisons. **a** Norway population (601 bp) **b** UK population (601 bp) **c** The Netherlands (323 bp). **d** WNA population (323 bp)

hunters (Shapiro et al. 2004; Drummond et al. 2005), and elephant seals responding to the gain and loss of breeding habitat with changing ice cover on the Antarctic mainland (DeBruyn et al. 2009). Spatial expansions may have occurred several thousands of years after the demographic expansion, though the distributions for the spatial expansion analyses were broad and poorly defined.

There are limitations to these interpretations (e.g. both selection and stochastic processes can affect the mismatch distributions), however a sudden demographic expansion,

together with a spatial expansion as suggested here for *L. albirostris*, has also been proposed for a number of other marine species (e.g., Wares 2002; Gysels et al. 2004; Adams et al. 2006; Costedoat et al. 2006; Haney et al. 2007). This has been attributed to climatic changes that took place during the Pleistocene Epoch, which may have generated several episodes of range expansions and contractions, with subsequent fluctuations in population sizes in various species (Taberlet et al. 1998; Hewitt 2000). During glaciated epochs changes in the sea level,

Table 4 Demographic and Spatial expansion statistics, and expansion times ($t = \tau/2\mu$) calculate from mtDNA control region

Sample	τ	SSD	SSD P-value	r	R P-value	$T(\mu_1)$	$T(\mu_2)$	CI 95%	$T(\mu_1)$ 95% CI	$T(\mu_2)$ 95% CI
Demographic expansion										
Norway (601 bp)	1.7	0.013	0.476	0.035	0.528	19708	2759	0.17 –10.77	2068–127953	290–17913
Norway (323 bp)	0.0	–	0.000	0.052	1.000	–	–	–	–	–
UK (601 bp)	5.2	0.046	0.145	0.114	0.234	62277	8719	0.078–9.88	929–117457	130–16444
UK (323 bp)	2.5	0.014	0.338	0.067	0.662	54622	7647	0.070–4.28	1555–94719	218–13260
The Netherlands	2.6	0.041	0.177	0.154	0.237	58138	8139	0–4.45	0–98386	0–13774
WNA	3.1	0.007	0.764	0.029	0.880	67552	9457	1.43–5.87	31617–129748	4426–18164
Spatial expansion										
Norway (601 bp)	1.3	0.013	0.436	0.035	0.545	15450	2163	0.47–8.58	5597–101930	783–14270
Norway (323 bp)	2.5	0.006	0.816	0.052	0.890	55285	7740	0.54–5.54	11935–122667	1671–17173
UK (601 bp)	3.3	0.050	0.085	0.114	0.518	39220	5491	0.74–8.97	8829–106578	1236–14921
UK (323 bp)	2.2	0.008	0.469	0.067	0.703	48651	6811	0.50–4.14	11145–91543	1560–12816
The Netherlands	2.3	0.025	0.252	0.154	0.449	50862	7121	0.50–4.82	11077–106625	1551–14927
WNA	2.5	0.007	0.784	0.029	0.893	55285	7740	1.15–5.12	25471–113346	3566–15868

$\mu_1 = 7.0 \times 10^{-8}$, Harlin et al. (2003); $\mu_2 = 5.0 \times 10^{-7}$, Ho et al. (2007); SSD, Sum of square deviations; r, Raggedness index

temperatures, upwelling patterns and prey distribution may have played an important role in the connection and isolation of populations (c.f., Costedoat et al. 2006; Harlin-Cognato et al. 2007). Therefore, it is possible that this led to a dynamic pattern of population colonisation and expansion, and a complex genetic signal of population founding and recovery in *L. albirostris*. A later expansion north along the Norwegian coast to the Barents Sea could have been a consequence of the spatial expansion further south, followed by a founder event across the North Sea, given the propensity for this species to occupy coastal habitat (though sometimes found some distance from shore, see Evans and Hammond 2004). The idea that the Norwegian-Barents Sea population could have been founded from the British Isle-North Sea population is supported by the more recent TMRCA calculated for this pair of putative populations, and the pattern of haplotype frequencies seen in the network (Fig. 3). However, intermediate populations across the North Atlantic and parts of the European distributional range were not included in this study, so a clear picture of the route of dispersal for this species could not be fully addressed.

Population structure

We found evidence for three *L. albirostris* populations in the North Atlantic: one in the NW Atlantic (though the sample size was too small to fully characterise this population's relationship to the others), one continuous population around the British Isle and in the North Sea and one population in the coastal and shelf waters of North Norway and the Barents Sea. The distinction between the latter two

populations was confirmed by both mtDNA and micro-satellite DNA markers.

These results are in agreement with previous cranio-metrical analyses published by Mikkelsen and Lund (1994), who found evidence for a least two separate populations of *L. albirostris* in the North Atlantic, one in the western North Atlantic (samples mainly from USA coast) and the other in the eastern North Atlantic (samples mainly from the North Sea). Levels of differentiation found between the samples from UK and Norway at microsatellite loci were similar to that found between different coastal populations of *L. obscurus* (i.e. South Africa versus Argentina; Cassens et al. 2005) and populations of *Phocoena spinipinnis* within Peruvian waters (Rosa et al. 2005). At the mtDNA locus levels were similar to that seen among populations of bottlenose whales (*Hyperoodon ampullatus*) between the eastern (Iceland) and western North Atlantic (Dalebout et al. 2006).

Conclusions

These analyses have provided some important implications for effective management. The phylogenetic data suggest the recovery of shallow variation after some variation was lost, possibly during a population contraction in refugial habitat during the last glacial epoch. The population genetic data suggest that comparatively short expanses of open water (across the English Channel) are crossed (either during the expansion phase, or continuously via ongoing migration), which may mean that contiguous coastline habitat could be managed as a single population, though

more inclusive sampling would be required to verify this. These data also showed that a more substantial distance across open water (between the British Isles-North Sea and northern Norway-Barents Sea) was apparently not crossed during the initial expansion phase (though it is not clear why contiguous populations along the southern Norwegian coast were not established). Instead, there is a signal for a later expansion along the Norwegian coast following a founder event. Though the data are few, it is also apparent that the WNA population is isolated from the ENA populations. Together these data emphasize the importance of coastal habitat to this species, and identify at least three separate populations that should be managed separately.

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