ELSEVIER

Biological Conservation 108 (2002) 175–182

BIOLOGICAL CONSERVATION

www.elsevier.com/locate/biocon

Mitochondrial genetic diversity and population structuring of UK bottlenose dolphins (*Tursiops truncatus*): is the NE Scotland population demographically and geographically isolated?

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Received 24 February 2001; received in revised form 1 March 2002; accepted 1 March 2002

Abstract

Concern has been expressed over the status of the bottlenose dolphin population that uses the Moray Firth and adjacent waters in NE Scotland. Consequently, part of this population's range has been proposed as a Special Area of Conservation. Efforts to manage and monitor the status of this population require information on the level of genetic diversity within the population and its genetic relatedness to neighbouring populations to appropriately designate units for management and monitoring. Here we examine mitochondrial genetic diversity within the NE Scotland population, and compare this to other regions around the UK and Ireland. Sequence analysis of 549bp of the mitochondrial DNA control region identified eight unique haplotypes in a sample of 29 individuals. Analysis of molecular variance suggests that the Moray Firth population is genetically more closely related to Welsh animals than to its nearest neighbour population in west Scotland. Furthermore, measures of within-population genetic diversity were markedly lower in the Moray Firth than any other sampled region. The low levels of mtDNA genetic variability observed and its apparent geographic isolation provide further support for the precautionary approach currently being applied to the management of this population, despite the lack of direct evidence of harm.

Keywords: Cetacean; Genetic diversity; *Tursiops truncatus*; mtDNA; Conservation

1. Introduction

Over the last 30 years, observations from dedicated surveys (Hammond et al., 1995; Berrow et al., 1996; Wood, 1998; Wilson et al., 1999; Grellier, 2000) and networks of volunteers (Evans, 1992) have indicated that bottlenose dolphins (*Tursiops truncatus*) are currently widespread along the south and west coasts of the UK and Ireland, but are more patchily distributed elsewhere around the UK (Fig. 1a). At present, the Moray Firth, and the surrounding waters of NE Scotland, are the only areas where this species regularly occurs in the North Sea, with Cardigan Bay (Wales) forming the main stronghold for the species along the west coast of the UK. In contrast to this contemporary discontinuous distribution, historical stranding records (Fig. 1b) indicate a relatively continuous distribution along the south and east coasts of England in the early part of the 20th century. Comparison of these contemporary and historical distributions suggest a contraction in the distribution of the extant North Sea population.

In recent years, such observations have led to concern over the status of bottlenose dolphins in UK waters (Evans, 1987; Simmonds, 1994; Thompson et al., 2000). In NE Scotland, direct individual-based photo-identification data suggest that only ca. 130 individual dolphins use the waters in and around the Moray Firth, and that there is no evidence of contemporary exchange with other studied groups in UK waters outwith NE Scotland (Wilson et al., 1999; P. Thompson, unpublished data). Furthermore, recent modelling work suggests that this small population may be in decline (Saunders-Reed et al., 1999), re-enforcing the need for conservation action. Similarly, studies in Cardigan Bay also indicate

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0006-3207/02/$ - see front matter
PII: S0006-3207(02)00103-9

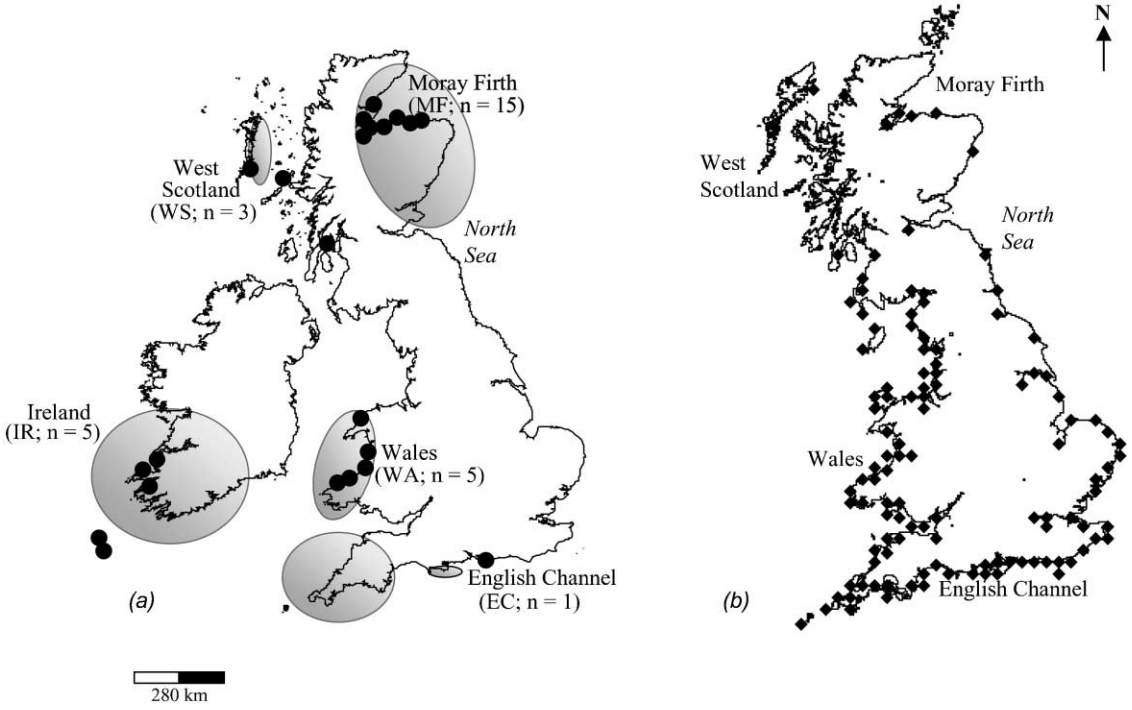

Fig. 1. Map of UK and Ireland illustrating (a) the contemporary distribution of bottlenose dolphins around the mainland UK and Ireland (ellipses indicate core areas of dolphin ranges), circles indicate location of samples, and (b) the distribution of strandings, 1913–1992 (Harmer 1914, 1915, 1916, 1917, 1918, 1919, 1921, 1923, 1925, 1927; Fraser 1934, 1946, 1953, 1974; Sheldrick 1976, 1989; Sheldrick et al. 1994). Diamonds represent locations of stranding events ($n \geqslant 1$).

that the number of animals frequenting Welsh coastal waters numbers less than 200 (Grellier et al., 1995).

In response to these concerns, the inner Moray Firth and parts of Cardigan Bay have been designated as candidate Special Areas of Conservation (cSAC) for bottlenose dolphins under the European Union Habitats Directive. As a result, management plans are being developed to reduce potential threats to the dolphins using these areas. Of particular concern for the NE Scotland population are activities such as dumping of contaminated harbour dredgings, pipeline construction, commercial fishing and dolphin-watching activities (Thompson et al., 2000) in the narrow inshore channels that are intensively used by bottlenose dolphins (Wilson et al., 1997). However, these threats are likely to be dispersed and it can be extremely difficult to obtain empirical evidence of cause and effect, thereby constraining efforts to manage human activities that may affect these populations. Predictive models suggest that the NE population may be in decline (Saunders-Reed et al., 1999), highlighting the need for precautionary measures that moderate activities despite a lack of evidence of harm before taking management measures (Thompson et al., 2000). Nevertheless, whilst these modelling scenarios support the proposed precautionary management of SACs, they depend critically upon assumptions about population structuring; notably that bottlenose dolphins in the NE of Scotland are isolated from groups occurring along the west coast of Britain and Ireland. Despite growing numbers of field studies of this species in UK waters, it can be difficult to detect contemporary movements of individually recognisable animals, and other sources of evidence must be explored to assess population structuring over different temporal and spatial scales.

The degree of genetic variability characteristic of the mitochondrial DNA (mtDNA) control region has been widely exploited in studies of population structure (Avise, 1994) and can be useful in identifying meaningful population subdivisions (Moritz, 1994); an important prerequisite to effective conservation, management and monitoring. Such analyses of mitochondrial sequence data have already proved invaluable in examining population differentiation in a number of cetacean species (e.g. Brown-Gladden et al., 1997; Walton, 1997; Pichler et al., 1998). In this study, we compare sequence information from a 549bp section of the mitochondrial control region of bottlenose dolphins from the NE Scotland population, and four other geographical regions throughout UK and Irish waters to assess the degree of isolation of the NE Scotland population as reflected by the geographic distribution of mtDNA variation. Conventional measures of genetic diversity are employed to assess the degree of genetic variation among samples within each of the geographic regions, and geographic subdivisions are examined among these NE Atlantic populations of bottlenose dolphins.

2. Methods

Information on the identity and location of cetaceans that have stranded around the UK coast has been recorded by the British Museum of Natural History since 1914 (Harmer, 1914, 1915, 1916, 1917, 1918, 1919, 1921, 1923, 1925, 1927; Fraser, 1934, 1946, 1953, 1974; Sheldrick et al., 1994). Our analysis of these stranding records indicates a significant northerly trend in the location of strandings found between 1914 and 1995 (linear regression of latitude against date of stranding, $F_{1,44} = 15.1$, $P < 0.001$, $r^2 = 0.26$). Although records for some species may be biased towards more populated coastlines, this programme provides a systematic reporting scheme that was extended to include post-mortem analyses and collection of tissue samples in 1990 (DEFRA funded UK marine mammal strandings project, contract CRO 238).

The samples used in this study were collected between 1988 and 1998, from 29 dolphins that were stranded or by-caught within five of the geographical regions currently frequented by bottlenose dolphins (Fig. 1a); NE Scotland (hereafter referred to as the Moray Firth: MF; $n = 15$), English Channel (EC; $n = 1$), Wales (WA; $n = 5$), Ireland (IR; $n = 5$), and West Scotland (WS; $n = 3$). Table 1 provides data on the temporal distribution, size and sex structure of the samples, and shows that samples were available from a reasonably representative sample of animals. The unequal sample size from each of the putative populations reflects the inevitable variation in the number of recovered animals in each region in recent decades (Sheldrick et al., 1994). Skin samples were obtained from archived tissues collected post-mortem and were preserved either in a 20% DMSO/ 5 M NaCl solution (Amos and Hoelzel, 1991), or frozen. Samples were also obtained from a striped dolphin (*Stenella coeruleoalba*) to provide a taxonomic outgroup in phylogenetic analyses.

Table 1
Summary information on the samples of by-caught and beach-cast bottlenose dolphins used in the analysis of mtDNA diversity and population structure ("n/a" denotes unavailable data)

Geographic region	Sex	Length (cm)	Date found (dd/mm/yy)	Latitude (N)	Longitude (W)
Moray Firth	F	148.00	18/8/89	57.520	4.210
Moray Firth	M	312.00	25/1/93	57.669	2.999
Moray Firth	M	325.00	2/2/93	57.909	3.995
Moray Firth	M	274.00	6/5/96	57.701	2.901
Moray Firth	M	275.00	23/4/94	57.533	4.105
Moray Firth	F	165.00	31/5/93	57.508	4.229
Moray Firth	M	147.00	12/8/95	57.574	4.089
Moray Firth	F	n/a	23/8/94	57.604	4.102
Moray Firth	F	134.00	11/8/92	57.704	3.482
Moray Firth	F	141.00	2/10/93	57.672	4.220
Moray Firth	M	128.00	11/10/92	57.687	2.931
Moray Firth	M	289.00	31/12/95	57.772	3.889
Moray Firth	M	145.00	7/12/93	57.943	3.999
Moray Firth	M	236.00	31/7/88	57.760	3.899
Moray Firth	M	236.00	31/7/88	57.760	3.899
English Channel	F	n/a	7/10/91	50.783	0.681
Wales	M	n/a	17/4/91	52.494	4.040
Wales	M	n/a	22/7/91	52.272	4.184
Wales	F	n/a	13/9/91	53.262	4.099
Wales	F	n/a	15/9/93	52.534	4.079
Wales	M	n/a	24/10/93	52.129	4.543
Ireland	n/a	n/a	25/8/93	52.578	9.755
Ireland	n/a	n/a	24/7/97	52.578	9.754
Ireland	n/a	n/a	11/3/98	52.700	9.700
Ireland	n/a	n/a	13/9/96	50.967	12.066
Ireland	n/a	n/a	13/9/96	50.967	12.067
W. Scotland	M	290.00	25/1/98	57.206	6.014
W. Scotland	F	322.00	1/3/94	56.017	4.800
W. Scotland	M	330.00	20/11/97	57.104	7.307

2.1. DNA extraction and sequencing

Total genomic DNA was extracted from skin tissue using standard proteinase K digestion, phenol/chloroform extraction procedures (Sambrook et al., 1989). Selective amplification of the mitochondrial DNA (mtDNA) fragment was performed using the polymerase chain reaction (PCR) primers L15926* (5′-ACACCAGTCTTGTAAACC-3′; Eggert et al., 1998) in the Thr-tRNA region, and H16498 (5′-CCTGAAGTAAGAACCAGATG-3′; Rosel et al., 1995). These primers amplify 549bp of the hypervariable 5′ section of the mitochondrial control region or D-loop which has been found to be highly variable in several cetacean species (see Pichler et al., 1998) including the bottlenose dolphin (Hoelzel et al., 1998). All PCR amplifications were carried out in 50 μl volumes containing 50 ng of template DNA, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.5 μM of each primer, and 2.5 units of Taq polymerase (Bioline). The PCR profile consisted of 35 cycles of 30 s denaturation at 92 °C, 30 s annealing at 60 °C, and 1 min primer extension at 72 °C, followed by 5 min elongation time at a constant 72 °C. Negative control reactions were included in each PCR run. PCR products were purified using QIAquick PCR purification columns (Qiagen). Both strands of the amplified PCR product were sequenced on an ABI 377 automated DNA sequencer using the BigDye sequencing kits (PE Biosystems).

The 29 control region sequence chromatograms were edited using the program CHROMAS V1.4 (McCarthy, 1997). The CLUSTALV program (Higgins et al., 1992) was used to align multiple sequences to identify polymorphic nucleotide sites and assign haplotypes.

2.2. Analysis of mtDNA sequence variation

Control region sequence variability within each population was tested for selective neutrality among mutations using Tajima's test, where large absolute

values of D indicate a deviation from neutrality (Tajima, 1989). Tajima's D statistic was calculated for the three populations for which $n \geqslant 4$ using the program DnaSP (Rozas and Rozas, 1999). Both nucleotide (π) and haplotype (h) diversities ($\pm$SE) for each putative population were estimated according to Nei (1987).

The amount of genetic variance explained by the a priori geographical structuring of the four populations for which $n > 1$ (MF, WA, IR and WS), was examined using an analysis of molecular variance (AMOVA; Excoffier et al., 1992). AMOVA analyses were performed both on the haplotype frequency data alone (F_{ST}), and on data incorporating information on the molecular diversity [calculated using the Tamura–Nei metric ($\alpha = 0.99$)] among pairs of haplotype sequences (Φ_{ST}) using the program ARLEQUIN (Schneider et al., 1997). Significance of the estimated Φ_{ST} and F_{ST} values (Weir and Cockerham, 1984) were tested by permutating the haplotypes among populations 1000 times using the non-parametric permutation procedures established in the ARLEQUIN software. In addition, pairwise population F_{ST} and Φ_{ST} statistics were calculated to investigate the genetic relationships among the putative populations.

3. Results

3.1. Sequence diversity

Twenty-one polymorphic sites were detected among the 29 individual samples, defining eight unique haplotypes (Fig. 2). Consistent with the transition bias reported among other cetacean species (Hoelzel et al., 1991; Walton, 1997), 19 of the 21 (90.5%) mutations were transitions. Tajima's test for neutrality provided no evidence of selection acting on this fragment ($P > 0.10$; Table 2). The eight control region haplotypes were distributed throughout the five geographic regions with the commonest (haplotype A) occurring in all of the four putative populations for which $n > 1$. Two of the haplotypes (haplotypes B and E) were represented by samples from two populations. The remaining five haplotypes were found only in samples from a single geographic region (Fig. 2). Although caution must be used when interpreting the geographic discreteness of these haplotypes due to the small sample sizes in the non-MF regions, it is worth noting that the EC sample had haplotype B, one of the haplotypes found in MF.

Overall, haplotypic diversity (h) among the 29 samples was estimated to be 0.697, though differences between populations were apparent (Table 2). These geographical differences in genetic diversity were also reflected in estimates of nucleotide diversity (π), which ranged from 0.0009 to 0.0164, with the level of nucleotide diversity in the NE Scotland population an order or magnitude less than that of the other regions (Table 2).

3.2. Geographical structuring of mtDNA variation

The amount of variation in the mtDNA control region sequences that can be explained by the geographic structuring of samples among the four populations, where sample sizes were greater than one (MF,

	Sequence	Frequency within geographic populations :				
	122233333 3333344445 5 3402501157 7888901680 0 4380944555 9789243701 2	MF	EC	WA	IR	WS
haplotype **A**	CTCTCTCTCA CTTCCCGTCC A	10		3	1	1
haplotype **B**	....T .	5	1			
haplotype **F**	.........G ....T .			1		
haplotype **I**	.CTCT...TC T.C.T.ACTT C				1	
haplotype **D**	T.T....C.. ..C.....TT C					1
haplotype **H**	T.T...TCT. ..CT.T..T. C				1	
haplotype **E**	T.T....CT. .CCT.T..T. C				2	1
haplotype **G**	T.T..C.CT. ..CT....T. C			1		

Fig. 2. Eight mitochondrial control region haplotypes. Variable sites are identified within the 549bp sequence where a "." represents identity with haplotype A (GenBank accession no. AF268357). The frequency of occurrence of each haplotype in each of the five sampled regions is also provided.

Table 2
Within-population measures of mitochondrial genetic diversity. Sample size (n), within-population nucleotide (π) and haplotype (h) diversities, and Tajima's D (Tajima, 1989) for each of the five geographic regions sampled

Sampling region	n	π ($\pm$SE)	h ($\pm$SE)	Tajima's D (absolute values)
Moray Firth	15	0.0009 ($\pm$0.0002)	0.476 ($\pm$0.092)	1.122^{NS}
English Channel	1	–	–	–
Wales	5	0.0080 ($\pm$0.0038)	0.700 ($\pm$0.218)	1.200^{NS}
Ireland	5	0.0164 ($\pm$0.0048)	0.900 ($\pm$0.161)	0.462^{NS}
West Scotland	3	0.0134 ($\pm$0.0042)	1.000 ($\pm$0.272)	–

Table 3
Results of analysis of molecular variance (AMOVA)[a]

Source of variation	d.f.	Sum of squares	Variance components	% of total variance
Among populations	*3*	*1.75*	*0.05*	*12.64*
	3	**25.68**	**1.16**	**41.35**
Within populations	*24*	*7.53*	*0.31*	*87.36*
	24	**39.53**	**1.65**	**58.65**
Total	*27*	*9.29*	*0.36*	
	27	**65.21**	**2.81**	
Fixation Index	$F_{st}=0.13$	$P=0.01$		
	$\boldsymbol{\Phi_{st}=0.41}$	$\boldsymbol{P<0.001}$		

[a] F_{st} values are based on haplotype frequencies alone and are represented by values in italics. Φ_{st} values are based on combined data of haplotype frequencies and molecular sequence diversity, these values are presented in bold typeface.

WA, WS, IR), was examined using an analysis of molecular variance (Table 3). Analysis using haplotype frequency data alone showed significant differences in the geographic distribution of molecular variance ($F_{ST}=0.13$, $P<0.01$). The inclusion of data on the molecular diversity among haplotypes increased levels of significant genetic differentiation between geographically-defined populations ($\Phi_{ST}=0.41$, $P<0.001$); this shows that 41% of the total genetic variance observed was due to interpopulation differences.

Based on haplotype frequencies (F_{ST}), significant differences were found between the Moray Firth and Ireland populations ($P<0.01$). When molecular diversity data among haplotypes was included, significant levels of genetic differentiation were found in pairwise population comparisons both between the Moray Firth and Ireland ($P<0.01$) and between the Moray Firth and west Scotland ($P=0.02$; Table 4). The results of the AMOVA suggest a significant degree of genetic structuring among the sampled populations, and significant levels of genetic differentiation between the NE Scotland (MF) and the northern west coast populations (WS and IR). Consequently, the dolphin population that inhabits the waters of NE Scotland appears to be more similar genetically to the Welsh population (WA) than to its current nearest neighbour on the west coast of Scotland (WS).

4. Discussion

Recent surveys suggest that the contemporary distribution of NE Atlantic bottlenose dolphins is discontinuous around the UK and Ireland, particularly within the North Sea where the species is recorded regularly only in the Moray Firth and adjacent coastal waters (Wilson et al., 1999). In this study, analysis of the distribution of mitochondrial genetic variance from samples of stranded or by-caught dolphins provides evidence for geographic structuring of populations around the UK. Notably, sequence variation suggests that the Moray Firth population is genetically more similar to the Wales population rather than the west Scotland population, its nearest neighbour geographically. Direct, field-based studies of identifiable individuals indicate that bottlenose dolphins exhibit relatively high degrees of residency in certain core areas such as the Moray Firth (Wilson et al., 1999), Cardigan Bay, Wales (Grellier et al., 1995), and the Shannon Estuary, Ireland (Ingram, 2000). Inevitably, in a species such as this, both direct studies based on photo-identification and genetic studies tend to be based on relatively small numbers of observations or samples. Nevertheless, these two sources of information indicate that currently, the NE Scotland population is both demographically and geographically isolated.

Table 4
Comparisons of pairs of population samples[a]

	Moray Firth	*Wales*	*Ireland*	*West Scotland*
Moray Firth		0.05 ($P=0.41$)	0.27 ($P=0.00$)	0.17 ($P=0.06$)
Wales	**0.15 ($P=0.09$)**		0.09 ($P=0.26$)	−0.03 ($P=0.57$)
Ireland	**0.67 ($P<0.01$)**	**0.22 ($P=0.07$)**		−0.180 ($P=0.99$)
West Scotland	**0.69 ($P=0.02$)**	**0.04 ($P=0.39$)**	**−0.21 ($P=0.99$)**	

[a] F_{st} values based on haplotype frequencies are presented in the upper matrix and Φ_{st} values based on both haplotype frequencies and molecular diversity (Tamura–Nei metric, $\alpha=0.99$) are presented in the lower matrix in bold typeface. Significance levels are based on 1000 permutations of the data.

Many indirect sources of information have pointed to a range contraction of this species in the southern North Sea over the last century (Kayes, 1985), but it is not known whether this is a result of a change in distribution or an overall decline in abundance. Bottlenose dolphins that previously mixed more freely with groups in the English Channel may have re-distributed into the northern North Sea in recent years. The geographic distribution of the eight mtDNA haplotypes further support this divergence between the North Sea population and those inhabiting the coastal waters of NW Scotland. Due to the natural variation in the number of incidentally obtained samples, the small sample sizes for some regions could potentially introduce bias into our estimates of genetic divergence. The low sample sizes of four out of the five sampled regions means that our statistical power to detect differences in the genetic variability among regions is low (Taylor et al., 1997; Tolley et al., 1999). However, it is important to note that small sample sizes generally tend to cause population differences to be statistically nonsignificant (Waples, 1998), resulting in an underestimate of the degree of population structuring.

Molecular diversity within the mtDNA control region sequences examined also revealed marked differences in the amount of intrapopulation genetic diversity. The 15 samples representing the Moray Firth population, our largest set of samples, comprised only two haplotypes that differed by a single nucleotide base (0.18%). This extremely low level of genetic diversity within the mitochondrial control region ($\pi=0.0009\pm0.002$) is an order of magnitude lower than any of the other regions sampled within this study (despite the much smaller sample sizes in these other areas), and many other studies of within-population genetic diversity of small cetaceans (e.g. Dowling and Brown, 1993; Walton, 1997; Pichler et al., 1998; Garcia-Martinez et al., 1999; Tolley et al., 1999). Studies by Hoelzel et al. (1998) and Wang et al. (1999) also examined phylogeographic patterns of mtDNA variation in *Tursiops truncatus*, however, the objective of these studies were for phylogenetic purposes and therefore 'populations' were defined with respect to broad geographical regions.

To the best of our knowledge, the only small cetacean populations studied to date that exhibit less genetic diversity are the North Island population of Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand, which currently consists of only a single matriline (Pichler and Baker, 2000), and the critically endangered vaquita (*Phocoena sinus*; Rosel and Rojas-Bracho, 1999). However, a lack of variability in the mitochondrial control region does not necessarily reflect low levels of genetic heterozygosity in the nuclear genome (Rosel and Rojas-Bracho, 1999). If a population has always been at relatively low numbers it is possible that it is able to persist as such, and not experience the negative effects of low genetic variability and inbreeding (Templeton, 1987; Pope, 1996). The relatively widespread historical pattern of strandings down the east coast of the UK, and the fact that the mtDNA sequence data suggest that this population is not significantly divergent from the population in Wales, imply that the Moray Firth population has not always been geographically isolated despite the species' relatively patchy contemporary distribution (Fig. 1a). Therefore, it is plausible that the contraction of the species' local distribution or recent declines in abundance (e.g. Pichler and Baker, 2000) have led to a decline in the genetic diversity of this currently isolated population.

However, evidence of geographical subdivision from mtDNA loci does not preclude the existence of male-biased dispersal and gene flow among these geographical populations (Palumbi and Baker, 1994; Avise, 1995). Nevertheless, because population recruitment is dependent upon female reproductive success and the matrilineal population structure reflected by mtDNA data suggests that the NE Scotland population is demographically isolated from the nearest population (WS), these mtDNA data provide key information for defining biologically meaningful conservation and management units.

Examinations of historical levels of genetic diversity and analyses of nuclear diversity are now required to confirm both the patterns of geographic subdivision inferred from our studies of mtDNA divergence, and the relative levels of intrapopulation genetic diversity.

Nevertheless, these data provide further support for the precautionary approach that has been taken in the development of management plans for bottlenose dolphin SACs, with efforts being made to identify and reduce potential threats even in the absence of direct evidence of harm. This initial examination of the genetic diversity and structuring in relation to current and historic distributions highlights the difficulties involved in designing marine protected areas for such highly mobile marine mammals. If the Natura 2000 network of SACs is to conserve and restore such highly mobile populations, management schemes must be sufficiently flexible to protect individuals as they move throughout their natural range.

Acknowledgements

Thanks to I.A. Patterson, P. Jepson, E. Rogan and R. Penrose for providing tissue samples. We are grateful to S.B. Piertney and J.W. Durban for valuable comments on earlier drafts of this manuscript. We also thank the UK Department of the Environment, Transport and the Regions who fund the cetacean strandings project and H.M. Coastguard, Local Councils and the individuals who reported strandings. Laboratory work was funded by NSERC and British Council Chevening scholarships awarded to K.M. Parsons.

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