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Fine-scale population genetic structuring of bottlenose dolphins in Irish coastal waters

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Keywords

bottlenose dolphins; population structure; Special Area of Conservation; microsatellites; mitochondrial DNA; Shannon Estuary.

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Abstract

The identification of localized discrete populations is particularly important to the management and conservation of animal species, especially in the marine environment, where potential for dispersal is high but barriers to gene flow are often not clear. We investigated population genetic structuring of bottlenose dolphins *Tursiops truncatus* found along the west coast of Ireland, with particular attention to the Shannon Estuary, which is the only candidate Special Area of Conservation designated for this species in Irish waters. A genetic structure analysis using 62 biopsy samples from free ranging dolphins and 23 necropsies from stranded dolphins revealed fine-scale population structure among three distinct populations. The Shannon Estuary population appears to be genetically isolated from adjacent coastal areas, with the exception of four animals sampled from a small group of six dolphins that are now resident in Cork Harbour (south coast) indicating ongoing gene flow or recent dispersal between these two areas. A second genetically distinct aggregation was identified in the Connemara–Mayo region, where recent photo-identification studies have suggested that dolphins found in this area show a degree of site fidelity. We found moderate nuclear (15 microsatellites) and low mitochondrial (544 bp of the control region) gene diversity in dolphins using the Shannon Estuary and the Connemara–Mayo region, while dolphins that stranded along the coast showed markedly higher levels of gene diversity at both classes of markers. Specifically, these stranded dolphins formed a third genetically distinct cluster, which may be part of a larger pelagic population, as also suggested by the high levels of gene diversity. These results provide new insights into population structure of bottlenose dolphins in Irish waters and will aid future management and conservation of the species in the eastern North Atlantic.

Introduction

Animal populations of conservation priority are often small and may be restricted to localized geographic areas (Smith *et al*., 2006). For cetacean species (whales, dolphins and porpoises), it is important to identify such populations because they are particularly vulnerable to habitat and climatic changes (Simmonds & Isaac, 2007) and tend to be directly exposed to anthropogenic impacts (e.g. Rojas-Bracho, Reeves & Jaramillo-Legorreta 2006; Currey, Dawson & Slooten, 2009). Bottlenose dolphins *Tursiops truncates* (Tursiops spp.) are among the most studied delphinid species throughout their worldwide distribution, where an increasing amount of information has revealed varying degrees of population structuring (e.g. Baird *et al*., 2009; Rossbach & Herzing, 1999; Möller & Beheregaray, 2004; Natoli, Peddemors & Hoelzel, 2008) and a need for revision of the taxonomic status at the inter-specific level (Kingston & Rosel, 2004; Natoli, Peddemors & Hoelzel, 2004; Natoli *et al*., 2005; Charlton, Taylor & Mckechnie, 2006; Möller *et al*., 2008). Habitat and resource specialization have been suggested as key factors in determining local differentiation among populations (Hoelzel Potter & Best, 1998; Segura *et al*., 2006; Mendez *et al*., 2010), and such specialization appears to have occurred independently in different ocean basins (Tezanos-Pinto *et al*., 2009).

Bottlenose dolphins have a widespread but patchy distribution in the eastern North Atlantic, where several localized coastal populations have been documented, as for example in Ireland (the Shannon Estuary, Berrow, Holmes & Kiely, 1996; Ingram & Rogan, 2002), Scotland (the Moray Firth, Wilson, Hammond & Thompson, 1999), Wales (Cardigan Bay, Evans, Anderwald & Baines, 2003), France (Brittany and Normandy, Kiszka, Hassani &

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Pezeril, 2004) and Portugal (the Sado Estuary, Dos Santos & Lacerda, 1987). Patterns of distribution and habitat use have been studied to some extent in Irish and UK coastal areas (Wilson, Thompson & Hammond, 1997; Wood, 1998; Wilson, *et al*., 1999; Ingram & Rogan, 2002; Stockin, Weir & Pierce, 2006; Culloch & Robinson, 2008; O'Brien *et al*., 2009; Pierpoint *et al*., 2009), though population structure and levels of connectivity among different areas are still poorly understood. At the inter-regional scale, recent population genetic studies have shown significant segregation among the eastern North Atlantic, western North Atlantic and the Mediterranean Sea (Natoli *et al*., 2004) and at the intra-regional scale, between Scotland and other eastern North Atlantic areas, including Portugal, Spain and South of England (Parsons *et al*., 2002; Natoli *et al*., 2005). In contrast, the occurrence of a large pelagic population in the North Atlantic has been suggested by Querouil *et al*. (2007), who noted a lack of mitochondrial genetic differentiation between bottlenose dolphins from the Azores, Madeira and offshore areas in the north-west Atlantic.

Bottlenose dolphins in Irish waters are entitled to protection under the Irish Wildlife Act (39/1976 and 38/2000) and are included on Annex II of the EU Habitats Directive (43/1992) as a species whose habitat requires special measures of protection with the designation of SACs (Ingram & Rogan, 2002). Along the west coast of Ireland, the Shannon Estuary is considered a critical habitat for bottlenose dolphins, which harbours about 120–140 individuals (Englund, Ingram & Rogan, 2007; Englund, Ingram & Rogan, 2008). Dolphins have been reported in the estuary since the mid-19th century (Knott, 1997) and have been directly studied since the early 19th nineties (Berrow *et al*., 1996). Dedicated boat surveys and photo-identification carried out in the Shannon Estuary have revealed long-term site fidelity and a seasonal trend in abundance with a possible decrease in numbers during winter (Ingram & Rogan, 2002; Englund *et al*., 2007). Results from photo-identification surveys conducted in the estuary since 1996 and other coastal areas since 2001 have not resulted in any matches of individuals between the Shannon Estuary and elsewhere in Ireland, suggesting a degree of isolation (Ingram, Englund & Rogan, 2001; Ingram & Rogan, 2003; O'Brien *et al*., 2009). Currently, the Shannon Estuary is the only candidate Special Area of Conservation (cSAC) designated for this species in Irish waters. In contrast, bottlenose dolphins occurring around the Irish coast, outside the Shannon Estuary, are thought to be transient and highly mobile, with large-scale movements (up to 650 km) reported for dolphins occurring between the west, south and east coasts of Ireland (Ingram & Rogan, 2002; O'Brien *et al*., 2009). However, despite these large ranging movements, coastal animals also show inter-annual site fidelity. Specifically, a recent photo-identification survey in the Connemara–Mayo region has revealed some degree of site fidelity and the occurrence of a relatively large (171 ± 48 SE) community of coastally transient bottlenose dolphins (Ingram *et al*. 2009). Further studies among such aggregations could reveal significant adaptive differentiation to different habitats and/or genetic segregation, which would aid the identification of distinct management units of conservation interest. Thus, understanding whether bottlenose dolphins using the Shannon Estuary and Irish coastal waters represent a single or several distinct populations will play a major role in defining management requirements and for the conservation of the species in this region.

Here we present a population genetic study of bottlenose dolphins using the waters of western Ireland, with particular attention to the Shannon Estuary cSAC. Using a suite of 15 microsatellite markers and a 544 base pairs (bp) fragment of the mitochondrial (mt) DNA control region, we assess levels of genetic diversity in the sampled areas, test the hypothesis of genetic structuring (vs. panmixia) in Irish waters and evaluate the degree of genetic distinctiveness between dolphins from the Shannon Estuary and adjacent coastal areas.

Methods

A total of 98 individual skin tissue samples were obtained from free-ranging dolphins from the Shannon Estuary ($n = 55$) between 2000 and 2007, the Connemara–Mayo area ($n = 14$) during summer 2009 and from Cork Harbour ($n = 6$) during summer 2008, using a biopsy darting system (Krützen *et al*., 2002) (see approximate location in Fig. 1). Furthermore, dolphins of unknown origin that were found stranded dead along the west coast of Ireland ($n = 23$, including three dolphins found dead within the Shannon Estuary) between 1993 and 2009 were also sampled (Fig. 1). Sex of stranded individuals was recorded by inspection of the genital area and reproductive organs, while sex of free-

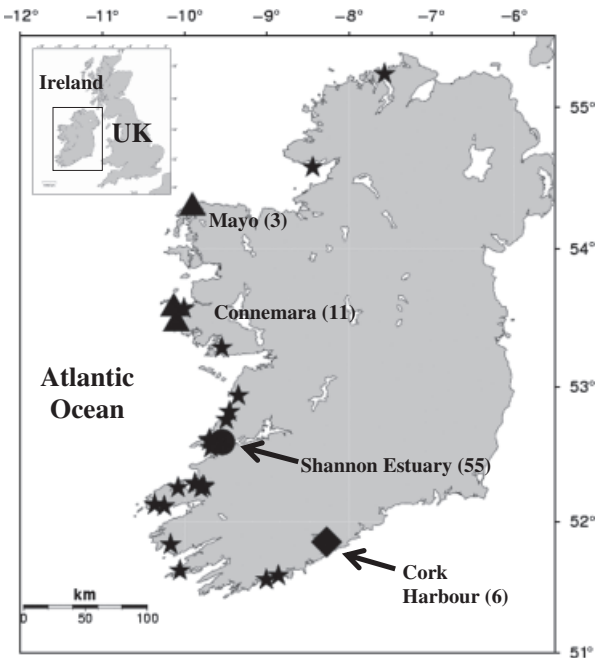


Figure 1 Map of Ireland showing sampling location of individually stranded dolphins (stars), Shannon Estuary (circle), Connemara–Mayo (triangles) and Cork Harbour (diamond). Numbers of multiple samples from the same area are shown in brackets.

ranging biopsied individuals was determined by multiplex amplification of sex chromosome-specific DNA fragments, following the method described in Rosel (2003).

A total of 15 nuclear microsatellite loci were amplified following polymerase chain reaction (PCR) conditions as described in the original publications (Table 1). Amplified products were separated on 6% polyacrylamide gels on a LI-COR 4300 DNA analyser (Li-Cor Inc, Lincoln, NE, USA) and allele sizes were determined by eye, by comparison to a 50–530 size standard (LI-COR) and allele cocktails from reference samples. In order to infer genotyping error rate, 15% (15 out of 98) of the available tissue samples were randomly chosen and re-processed (i.e. from DNA extraction to PCR amplification). Amplification of a portion of the mtDNA control region was carried out using primers L15926 and H00034 (Rosel, Dizon & Heyning, 1994). Sequencing of each PCR product was carried out using both primers by means of the Sanger method (Sanger, Nicklen & Coulson, 1977), using the DNA cycle sequencing kit (Jena Biosciences GmbH, Jena, Germany) following manufacturer instructions. Sequencing products were separated on 6% polyacrylamide gels using a LI-COR 4300 s DNA analyser. Some sequencing was also carried out commercially (GATC Biotech AG, Konstanz, Germany), using the same set of primers. For each individual, the sequence trace from the forward primer was aligned to its relative reverse-complementary trace using Codoncode Aligner 2.0 (CodonCode Corporation, Dedham, MA, USA) (http://www.codoncode.com/index.htm) and checked by eye.

For mtDNA sequence data, the number of haplotypes, and nucleotide and haplotype diversity were calculated using ARLEQUIN (Excoffier, Laval & Schneider, 2005). Each microsatellite locus was checked for the presence of potential genotyping errors caused by null alleles, large allele dropout and stuttering, using MICROCHECKER 2.2.3 (van Oosterhout *et al.*, 2004) and selecting the Bonferroni adjusted 95% confidence interval option (1000 simulations). Because sampling of free-ranging dolphins may lead to duplicate samples, multi-locus genotypes were compared between all pairs of dolphins. The probability of identity (PI) (Waits, Luikart & Taberlet, 2001) was used as an indicator of the power of the markers to resolve between two distinct individual samples and was calculated using the program GIMLET 1.3.3 (Valière, 2002). Number of alleles, allelic richness and the inbreeding coefficient F_{IS} (to test for non-random mating) and its significance [15000 randomizations and indicative adjusted nominal level (5%) of 0.00333] were calculated using FSTAT 2.9.3.2 (Goudet, 2001). Possible linkage (disequilibrium) between all pairs of loci was tested, levels of diversity were evaluated as observed and expected heterozygosity, and Hardy–Weinberg equilibrium tests were carried out to detect possible departure from equilibrium, using ARLEQUIN 3.1. Furthermore, populations that undergo a significant reduction in effective size (e.g. bottleneck) tend to show a sudden drop of the number of alleles per locus resulting in an excess of heterozygosity (Luikart *et al.*, 1998). In order to test for possible recent reduction in effective size of the Shannon population, we used BOTTLENECK 1.2.02 (Piry, Luikart & Cornuet, 1999) to run a one-tailed Wilcoxon test (10000 simulations) assuming default parameters of mutation-drift equilibrium and that all loci fit a two-phase mutation model (95% single step, 5% multiple step with variance equal to 12), as suggested for microsatellite loci by Piry *et al.* (1999).

Because barriers to gene flow tend to be not evident in the marine environment and because origin of some individuals (i.e. stranded animals) was unknown, we first investigated population structure with no prior information on sampling location, using the model-based approach implemented in STRUCTURE 2.2 (Pritchard Stephens & Donnelly, 2000). This approach sorts multi-locus genotypes into Hardy–Weinberg/linkage equilibrium clusters, hence assigning individuals to populations. Ten Markov Chains Monte Carlo (MCMC) runs were carried out for each of six *K*-values (1–6), using the admixture model and co-rrelated allele frequencies among putative populations. Convergence among independent runs was reached with medium-short runs of 100 000 (burn in) and 1 000 000 MCMC repetitions, and results from all runs were summarized and inspected using STRUCTURE HARVESTER v0.56.4 (Earl, 2009). Individual assignment to putative populations was then carried out by inspection of the proportion of membership (i.e. individual's estimated membership fraction to each of the *K* inferred clusters). Because population structure analyses can be affected by the presence of related individuals (i.e. family effect), we tested the hypothesis of no relatedness (Mathieu *et al.*, 1990) using IDENTIX 1.1 (Belkhir, Castric & Bonhomme, 2002). The maximum likelihood approach implemented in the program COLONY 2.0 (Wang, 2004) was used to identify potential groups of closely related individuals (full sibs or parent offspring). In order to evaluate the level of genetic differentiation among putative populations accounting for different mutation models, pairwise *F*-statistics were calculated based on genotypic and haplotypic frequencies (F_{ST} and R_{ST}) (Weir & Cockerham, 1984; Michalakis & Excoffier, 1996) and a corrected percentage of different nucleotides Φ_{ST} (Tamura & Nei, 1993) (10100 permutations), using ARLEQUIN.

Results

Molecular sexing allowed the determination of gender of all individuals except one (due to PCR amplification failure). Repetition of individual genotypes (from DNA extraction to amplification) indicated a negligible genotyping error rate (i.e. $<0.01\%$). The probability (PI) that two individuals shared the same genotype over the 15 microsatellite loci was 6.4^{-14} for any two random unrelated individuals and 6.9^{-6} for siblings, indicating that the set of markers have a high resolution power in discriminating identical genotypes that may have originated by chance alone. Subsequently, comparison of individual genotypes revealed that 11 dolphins (seven from Shannon, two from Cork, two from Connemara–Mayo) were sampled twice and one Shannon dolphin was sampled three times throughout the sampling period (between 2000 and 2009). In all instances, each dolphin was

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Table 1 Genetic variability at 15 microsatellite loci in the stranded (n=23), Shannon (n=46) and Connemara–Mayo (n=12) samples and for all samples (ALL, n=85), including the four Cork individuals

	Stranded					Shannon					Connemara–Mayo					ALL			
	N_a [private]	A_R	H_O	H_E	F_{IS}	N_a [private]	A_R	H_O	H_E	F_{IS}	N_a [private]	A_R	H_O	H_E	F_{IS}	N_a	H_O	H_E	F_{IS}
D18[a]	8 [4]	7.0	0.739	0.818	0.099	4 [0]	2.3	0.196	0.182	−0.076	4 [1]	3.8	0.500	0.598	0.170	9	0.376	0.498	0.245
D22[a]	10 [6]	7.9	0.783	0.833	0.062	4 [0]	3.4	0.558	0.590	0.055	3 [0]	3.0	0.545	0.567	0.040	10	0.630	0.728	0.136
*Dde*59[b]	8 [4]	7.4	0.682	0.842	0.194	4 [0]	3.9	0.800	0.702	−0.141	3 [0]	2.9	0.667	0.554	−0.214	8	0.747	0.766	0.025
*Dde*61[b]	5 [2]	4.8	0.739	0.763	0.032	3 [0]	3.0	0.682	0.597	−0.144	3 [0]	2.9	0.167	0.301	0.457	5	0.614	0.692	0.113
*Dde*65[b]	5 [2]	4.5	0.783	0.698	−0.123	4 [1]	3.5	0.558	0.480	−0.165	2 [0]	2.0	0.250	0.344	0.283	6	0.573	0.532	−0.079
*Dde*66[b]	10 [8]	6.9	0.545	0.635	0.144	2 [0]	1.9	0.186	0.171	−0.091	1 [0]	1.0	0.000	0.000	NA	10	0.259	0.298	0.131
*Dde*69[b]	5 [1]	4.9	0.826	0.742	−0.116	4 [0]	3.8	0.565	0.525	−0.078	3 [0]	3.0	0.417	0.420	0.009	5	0.623	0.602	−0.036
*Dde*72[b]	9 [4]	7.9	0.783	0.841	0.070	5 [0]	4.5	0.600	0.619	0.031	4 [0]	3.9	0.500	0.576	0.137	9	0.631	0.739	0.147
GATA098[c]	8 [4]	6.8	0.826	0.813	−0.016	4 [0]	3.9	0.744	0.708	−0.052	3 [0]	3.0	0.667	0.594	−0.128	8	0.744	0.775	0.041
*Ttr*04[d]	7 [3]	6.1	0.870	0.818	−0.064	3 [0]	3.0	0.674	0.609	−0.108	5 [0]	4.0	0.667	0.648	−0.029	7	0.729	0.724	−0.008
*Ttr*11[d]	10 [7]	7.6	0.870	0.852	−0.021	3 [0]	2.9	0.578	0.574	−0.006	3 [0]	2.9	0.417	0.359	−0.170	10	0.655	0.702	0.068
*Ttr*34[d]	6 [1]	5.4	0.727	0.782	0.072	3 [0]	2.9	0.667	0.564	−0.183	5 [0]	5.0	0.750	0.779	0.039	6	0.675	0.681	0.010
*Ttr*48[d]	7 [2]	6.4	0.818	0.839	0.026	4 [0]	3.9	0.696	0.649	−0.073	3 [0]	2.8	0.167	0.163	−0.023	7	0.643	0.685	0.062
*Ttr*63[d]	15 [7]	10.7	0.913	0.898	−0.018	9 [0]	7.3	0.935	0.855	−0.094	5 [0]	5.0	0.818	0.671	−0.233	16	0.905	0.888	−0.018
*Ttru*AAT44[e]	7 [3]	6.2	0.783	0.804	0.027	4 [0]	3.9	0.587	0.563	−0.044	3 [0]	3.0	0.333	0.583	0.439	7	0.583	0.614	0.120
	8.0	6.7	0.779	0.798	0.025	4.0	3.6	0.602	0.559	−0.077	3.3	3.2	0.458	0.477	0.043	8.2	0.626	0.662	0.059*
	(2.6)	(1.6)	(0.089)	(0.066)		(1.6)	(1.2)	(0.196)	(0.179)		(1.1)	(1.0)	(0.239)	(0.209)		(2.8)	(0.152)	(0.140)	
Mean (SD.)	[3.9 (2.2)]					[0.1 (0.3)]					[0.1 (0.3)]								

[a]Shinohara *et al.*, 1997.
[b]Coughlan *et al.*, 2006.
[c]Palsbøll *et al.*, 1997.
[d]Rosel *et al.*, 2005.
[e]Caldwell *et al.*, 2002.
*Significantly positive (P=0.0002).
N_a, number of alleles; Private, private alleles; A_R, allelic richness (based on a minimum sample size of eleven individuals); H_O, observed heterozygosity; H_E, expected heterozygosity; SD, standard deviation; F_{IS}, inbreeding coefficient, NA, not available.

re-sampled in close proximity to the original location of sampling, where time difference between sampling of the same individual ranged between 3 days to a maximum of 7 years. Thus, all replicate samples ($n = 13$) were excluded from subsequent analyses, which were carried out on a total of 85 distinct individuals (46 from the Shannon Estuary, four from Cork Harbour, 12 from the Connemara-Mayo area and 23 stranded dolphins), including 55 males, 29 females and one unknown gender.

No linkage between pairs of microsatellite loci and no evidence of null alleles or allelic dropout were detected. Overall, all loci were polymorphic with five to 16 alleles per locus, and observed and expected heterozygosity ranging between 0.217–0.917 and 0.242–0.874, respectively (Table 1). Levels of polymorphism varied among the sampling groups, with stranded dolphins showing highest allelic richness (6.7) and observed gene diversity (0.779), and a consistently higher proportion of private alleles across loci (47% of total number of alleles) (Table 1). No evidence of inbreeding (F_{IS}) and no departure from Hardy–Weinberg expected proportions were detected in any of the sampled groups. However, when pooling samples from all areas together, a significantly positive F_{IS} value (0.059, $P = 0.0002$) was found over all loci and a significant deviation from Hardy–Weinberg expected proportions was detected in 10 out of 15 loci (Table 1). No evidence of a recent reduction of effective population size (i.e. bottleneck) was detected (one-tailed Wicoxon test, $P > 0.05$). When analysing data with no prior information on sampling location, the STRUCTURE approach indicated that the data set was best explained by a three cluster (K) model, which was consistent in all repeated runs (i.e. highest likelihood values were always obtained for $K = 3$). Results from all STRUCTURE runs are summarized in supporting information. The proportion of membership of each individual genotype is shown as coloured vertical bars in Fig. 2, where most individuals were assigned to any of the three clusters that best explained the chosen model, given the observed data. Cluster one (white) comprised all but one (possible migrant) dolphin biopsied in the Shannon Estuary, the four Cork Harbour dolphins, the three dolphins that were found dead within the Shannon Estuary and two dolphins that were found dead within 100 km from the mouth of the estuary. Cluster two (grey) comprised all dolphins biopsied in the Connemara–Mayo area, one dolphin biopsied inside the Shannon Estuary, and two dolphins that stranded along the west coast. The third cluster (black) comprised most dolphins (16 out of 23) that stranded along the Irish coasts (see Fig. 2). When testing the hypothesis of no relatedness within the identified populations (IDENTIX), significant departure from mean and variance of unstructured populations was detected in the Shannon (mean $P < 0.001$, var. $P < 0.01$) and Connemara–Mayo (mean $P > 0.05$, var. $P < 0.05$) populations, while the group of stranded individuals conformed to proportions expected in unstructured populations. The COLONY approach indicated that most individuals within populations were not closely related to each other, however, larger numbers of potentially closely related individuals were identified in the Shannon and Connemara–Mayo populations with varying degrees of confidence (data not shown). Nonetheless, STRUCTURE was run with a reduced sample set and removal of potentially closely related individuals (up to 20) did not affect population structuring results (i.e. $K = 3$). Pairwise F_{ST} between the three identified populations are shown in Table 3, where significant genetic differentiation was detected among all areas, although it was more marked when using the number of alleles-based estimator (F_{ST}-like), than the sum of squared size difference-based estimator (R_{ST}-like).

A 544 bp fragment of the mtDNA control region was obtained from all but three individuals. Comparison of aligned consensus sequences allowed the identification of 36 polymorphic sites, including 33 transitions, two transversions and two indels, and a total of 14 different haplotypes (Ire1-Ire14, GenBank accession numbers: HQ634245-HQ634258) (Table 2). The overall nucleotide and haplotype diversities were 0.643 (± 0.048) and 0.011 (± 0.006), respectively. In particular, a large portion of variability was due to haplotype Ire9, which accounted for nine out of the 36 polymorphic sites (25%) and that was obtained from a dolphin that stranded in the north-west of Ireland (second star from the top in Fig. 1). Low haplotype diversity was found in the Shannon Estuary sample (0.279 ± 0.076), where only two different haplotypes (Ire1 and Ire2) were detected, with the most common haplotype (Ire1) found in 37 out of 44 individuals (Table 2). The four Cork dolphins presented the same two haplotypes, with Ire1 being the most common

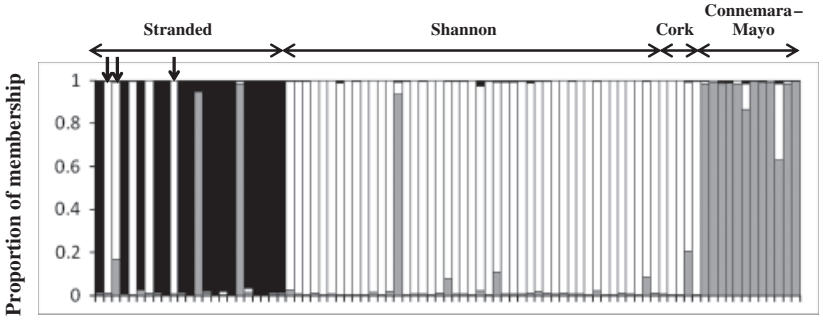


Figure 2 Proportion of each individual genotype (vertical bars) assigned to cluster one (black), cluster two (white) and cluster three (grey), using STRUCTURE. Arrows indicate three dolphins that stranded within the Shannon Estuary.

Table 2 MtDNA control region variability and haplotype frequency in the sampled areas and for the whole Irish sample (ALL), based on 544 bp (position one corresponds to position 15424 in *Lagenorhynchus albirostris* mitochondrial sequence, complete genome, GenBank Acc. No. AJ554061)

Haplotype	Polymorphic sites	Strandings	Shannon	Connemara–Mayo	Cork	ALL
	1 111 112 223 333 333 333 333 344 444 455					
	557 894 566 894 591 122 222 334 455 702 444 402					
	891 348 501 094 555 924 789 023 435 970 123 438					
	-GG TCC ATG CCT TCA CAC TTC TCT CGT CTC CAT CTC					
Ire1	... ..T ... ... CT. ... CCT ... T.. ..T .C. ..T	2	37	3	3	45
Ire2	... ..T ... ... C.. ... .C. ... .T TC. ..T	4	7	8	1	20
Ire3	... ..T ... ... CT. ... .CT ... ... T.T .C. .C.	3				3
Ire4	... ..T ... ... CT. ... .CT ... T.. ..T .C. ..T	2				2
Ire5	... C.T .C. .T. .TC T.. .C. .T. ... .CT TC. ...	2				2
Ire6	.A. ..T ... ... CT. ... CCT ... T.. ..T .C. ..T	2				2
Ire7	... ..n ... ... CT. ... .nT .T. n.. ..T .C. ..T			1		1
Ire8	..A ... G.A TT. CTG .-T ..T C.C T.. .CT TC. T..	1				1
Ire9	A.. .TT ... ... C.. ... .C. .T. ... ... TC. ...	1				1
Ire10	... ..T ... ..C CT. ... .CT ... .T .C. .C.	1				1
Ire11	... ..T ... ... CT. ... .CT ... .T TC. .C.	1				1
Ire12	... ..T ... ... CT. ... .CT ... ..C ..T TC. .C.	1				1
Ire13	... ..T ... ... CT. ... .CT ... T.. .CT .CC ..T	1				1
Ire14		1				1
Number of individuals		22	44	12	4	82
Number of haplotypes		13	2	3	2	14
Haplotype diversity		0.943 (±0.028)	0.274 (±0.076)	0.530 (±0.136)	0.500 (±0.265)	0.643 (±0.048)
Nucleotyde diversity		0.014 (±0.007)	0.005 (±0.003)	0.008 (±0.005)	0.010 (±0.007)	0.011 (±0.006)

Table 3 Estimates of pairwise population differentiation for mtDNA data (below diagonal) and microsatellite data (above diagonal)

	Shannon Estuary	Connemara–Mayo	Strandings
Shannon Estuary		$F_{ST}=0.179^{***}$	$F_{ST}=0.170^{***}$
	–	$R_{ST}=0.146^{**}$	$R_{ST}=0.062^{*}$
Connemara–Mayo	$F_{ST}=0.353^{***}$		$F_{ST}=0.177^{***}$
	$\varphi_{ST}=0.398^{***}$	–	$R_{ST}=0.095$
Strandings	$F_{ST}=0.432^{***}$	$F_{ST}=0.251^{***}$	–
	$\Phi_{ST}=0.457^{***}$	$\Phi_{ST}=0.177^{**}$	

$^{*}P<0.05$,
$^{**}P<0.01$,
$^{***}P<0.001$.
Significance levels are shown after Bonferroni correction.

(three out of four). Reduced gene diversity was also detected in the Connemara–Mayo dolphins (0.530 ± 0.136), which were characterized by the same two mtDNA variants (Ire1 and Ire2, although the latter was the most common), and a third unique haplotype (Ire7) (Table 2). In contrast, the sample of stranded dolphins showed greater genetic diversity (0.943 ± 0.028), with 13 different haplotypes found in 22 individuals (Table 2). Significant pairwise estimates of mtDNA differentiation were obtained between each of the three populations using both F_{ST} and Φ_{ST} estimators (Table 3).

Discussion

Genetic diversity

In the present study, levels of polymorphism varied among the sampled areas, where a higher nuclear and mitochondrial gene diversity was found in dolphins that stranded 'randomly' along the coast than dolphins biopsied in the Shannon Estuary, Cork Harbour and Connemara–Mayo area. In particular, the Shannon Estuary and Connemara–Mayo coastal dolphins were characterized by a moderate nuclear diversity and a very low mitochondrial haplotype diversity. This is in agreement with previous studies of bottlenose dolphins in the North Atlantic, which reported that coastal populations tend to show lower levels of genetic diversity than offshore populations (e.g. Parsons *et al.*, 2002; Natoli *et al.*, 2004; Nichols *et al.*, 2007; Querouil *et al.*, 2007). In contrast, small genetically distinct populations occurring in coastal waters around New Zealand showed an unexpectedly high mitochondrial diversity, which was suggested to be the result of long-distance dispersal, but could also reflect a recent founder event in populations that still retain historical levels of high variability (Tezanos-Pinto *et al.*, 2009). The particularly low mtDNA diversity found in coastal populations can be explained by several factors, such as genetic drift and differential philopatry between sexes. Genetic drift can rapidly affect diversity in small populations and tends to be stronger on the smaller effective population size of the haploid (maternal) mtDNA genome. On the other hand, loss of variability may be prevented by gene flow among populations, which may be characterized by sex-biased dispersal, as has been suggested in other marine mammal species (e.g. O'Corry-Crowe *et al.*, 1997; Lyrholm *et al.*, 1999; Escorza-Treviño & Dizon, 2000). In the case of bottlenose dolphins, no strong bias for dispersal was noted between sexes in areas encompassing the eastern North Atlantic, the Black Sea and the Mediterranean Sea, with the exception of some differential female movements at the margins of their range (Natoli *et al.*, 2005). In other regions, contrasting patterns of dispersal have been described for coastal populations of *Tursiops* spp. in Australian waters, with both sexes showing natal site philopatry in a population resident in the west coast (Connor *et al.*, 2000) and with females being the more philopatric sex in small coastal populations in the south east coast (Möller & Beheregaray, 2004). In the case of the Shannon Estuary, the exceptionally low mtDNA diversity may reflect female philopatry, such a hypothesis requires a better understanding on dispersal, population and social structure, which can be achieved by carrying out further photo-identification and molecular analyses.

A number of authors have suggested that coastal populations of bottlenose dolphins may originate from independent founder events, perhaps as a result of resource specialization or philopatry (Hoelzel *et al.*, 1998, Natoli *et al.*, 2004, Sellas, Wells & Rosel, 2005; Tezanos-Pinto *et al.*, 2009). Recent analyses of historical samples (from a now extinct aggregation that occurred at least 100 years ago in the Humber river estuary off the east coast of England) suggested local habitat dependence within a metapopulation model characterized by local extinction events (Nichols *et al.*, 2007). Interestingly, the Humber river population showed very similar levels of both nuclear and mitochondrial genetic diversity that were similar to those observed in the Shannon Estuary population (c.f. Nichols *et al.*, 2007). In the present study, no evidence of inbreeding or recent reduction of effective population size (i.e. bottleneck) could be detected in any of the sampled areas using nuclear microsatellite markers, indicating that these aggregations of dolphins have not suffered great losses of genetic variability and have been stable in recent generations. This is in agreement with historical and distributional data of Shannon Estuary bottlenose dolphins, where presence of the species has been reported as far back as the mid-19th century (Knott, 1997) and long-term site fidelity (Englund

et al., 2007) suggests the potential for local adaptation to the Shannon estuarine environment.

Population structure in Irish coastal waters

The analyses of nuclear and mitochondrial differentiation among the sampled areas revealed fine scale population segregation in coastal Irish waters and provided important insights into isolation of bottlenose dolphins populations where no obvious barriers to gene flow are present. Though strong genetic structuring was found with both nuclear microsatellite and mitochondrial markers, F_{ST}-like estimators appear to show stronger differentiation than R_{ST}-like estimators when using microsatellite data. This could be due to the mutation pattern of the loci used (e.g. not following either a Stepwise or two-phase mutation model) and/or to a tendency of R_{ST}-like estimators to produce larger variance (Slatkin, 1995). Genetic results presented here corroborate previous data on distribution and site fidelity in the sampled areas. Long-term site fidelity has been documented in the Shannon Estuary (Berrow *et al*., 1996; Ingram & Rogan, 2002; Englund *et al*., 2007; Englund *et al*., 2008) and in Connemara (Ingram *et al*., 2009) although short term and seasonal changes in use of these areas indicates animals are ranging well beyond the limits of survey effort. With the exception of Cork Harbour, dolphins occurring along the west and south coasts appear to be highly mobile with extensive coastal ranging behaviour (O'Brien *et al*., 2009). In contrast, Shannon dolphins are rarely encountered outside the lower River Shannon SAC (Ingram *et al*., 2001; Ryan & Berrow, 2011) and there is no evidence of extensive movements outside of the SAC (O'Brien *et al*., 2009), though factors that are driving such a discreteness are currently unclear.

The finding that animals stranded along the west coast of Ireland appear to originate from a third discrete population requires some consideration. The geographical origin of stranded dolphins is often unknown due to *post mortem* drifting with water currents (in case of death at sea) and unpredictable behaviour of dying dolphins (in case of live-strandings). However, the higher levels of both nuclear and mitochondrial diversity suggest that these dolphins may be part of a larger population, which may occur between coastal and continental shelf waters. In fact, bottlenose dolphins are known to be widely distributed in the eastern North Atlantic shelf waters, around the shelf edge and in the deeper waters of the Rockall Trough and on the Rockall Bank (P.S. Hammond *et al*., unpubl. data; Reid, Evans & Northridge, 2003; Macleod *et al*., 2008). Results presented here raise the hypothesis of the presence of a genetically distinct pelagic population to the west of Ireland, thus further studies on the identity and structuring of this putative offshore population should be carried out.

Varying degrees of genetic structuring have been previously described in the eastern North Atlantic, with Scottish bottlenose dolphins showing genetic isolation from other Atlantic areas (Parsons *et al*., 2002; Natoli *et al*., 2005), as well as other regions throughout the species distribution range (e.g. Baird *et al*., 2009; Hoelzel *et al*., 1998; Rossbach & Herzing, 1999; Wang, Chou & White, 1999; Natoli *et al*., 2005; Parsons *et al*., 2006; Segura *et al*., 2006; Querouil *et al*., 2007). The strong levels of population structuring reported in the present study are similar to those found in inshore resident populations in the Gulf of Mexico (Sellas *et al*., 2005) and New Zealand (Tezanos-Pinto *et al*., 2009), but are unprecedented on a small geographic scale in coastal waters of the eastern North Atlantic (Parsons *et al*., 2002; Natoli *et al*., 2005). Parsons *et al*. (2006) suggested that the scale of population subdivision in this species reflects the genetic consequences of their social system and site fidelity. In fact, social structure plays a very important role in shaping genetic variability within and between populations, since it determines patterns of grouping of related and unrelated individuals in space and time (Sugg *et al*., 1996; Storz, 1999). Thus, the presence of some closely related individuals, as shown by the IDENTIX and COLONY approaches, is not surprising given the levels of social cohesion reported in other coastal populations of bottlenose dolphins (reviewed in Connor *et al*., 2000). The lower genetic diversity found in both the Shannon and the Connemara–Mayo populations could be linked to the presence of such clusters of related individuals. However, removal of potentially closely related individuals appears not to significantly affect levels of diversity and population structure analyses carried out in the present study. Ongoing work on relatedness and group formation patterns within the Shannon Estuary should provide important insights on social structure patterns of this population, hence aiding interpretation and understanding of population structure in this area. It is important to point out that population structure and dispersal of marine species can be shaped by demographic and behavioural factors as well as ecological and environmental processes (Gaggiotti *et al*., 2009). For example, food availability has been suggested as one of the main factors affecting shifts in distribution and habitat use of bottlenose dolphins in another cSAC area (the first of its kind) in the Moray Firth, north-east Scotland (Wilson *et al*., 2004). Recently, Fernández *et al*. (2009) showed that dolphins from southern Galicia (Spain) were genetically distinct from northern Galician and offshore populations, which reflected differences in diet, stable isotope ratios and habitat preferences (Fernández, 2010). Thus, equal research effort should be allocated to the understanding of environmental, ecological and behavioural processes in the cSAC Shannon area in order to understand possible driving factors for distribution and occurrence of the species along the Irish coasts.

Genetic isolation of the Shannon Estuary population

The Shannon Estuary is the only cSAC designated for this species in Irish waters and results presented here further highlight a genetic isolation of Shannon bottlenose dolphins from adjacent coastal areas. Such isolation is in strong agreement with photo-identification data, which indicate

long-term inter-annual site fidelity in the estuary and no evidence of mixing with dolphins using other sites. In fact, only very limited movements of Shannon dolphins have been reported outside the estuary (Ingram & Rogan, 2002; Ryan & Berrow, 2011). The finding in the present study of two dolphins genetically assigned to the Shannon population that stranded not far out (<100 km) from the mouth of the estuary may suggest that some Shannon dolphins may range outside the estuary, although carcasses may drift out of the estuary *post mortem*. Although genetic data clearly shows segregation between the Connemara–Mayo and the Shannon dolphins, the identification of a possible migrant from the former to the latter area suggests limited but possible interactions between these two populations. Similar to the Shannon area, the Moray Firth cSAC has been established following research conducted in the 1980s and 1990s, in order to conserve the local resident population of bottlenose dolphins (Wilson *et al*., 1997; Wilson *et al*., 1999). Nonetheless, a range expansion of the population has been documented in more recent years possibly as a consequence of prey availability and distribution (Wilson *et al*., 2004). This indicates that long-term mobility should also be actively incorporated in management structures such as the European Union Habitat Community Directive cSACs (92/43/EEC). In fact, recent studies have shown that aggregations of dolphins occurred more frequently than previously thought in waters adjacent to the Moray Firth cSAC (Culloch & Robinson, 2008), as well as in more distant areas, such as Aberdeenshire's coastal waters (Stockin *et al*., 2006). Thus, while local populations may certainly benefit from the establishment of SACs, it is also extremely important to report potential changes in distribution and range in order to ensure the effectiveness of conservation regulations (Thompson *et al*., 2000; Wilson *et al*., 2004). In order to enhance the efficiency of management structures such as the Shannon Estuary cSAC, it is therefore recommended that movements and interactions of dolphins among this and other adjacent areas should be regularly monitored. Furthermore, following results from the present study, additional analyses (possibly using larger sample sizes) should be carried out to assess the extent of isolation of a putative population in the Connemara–Mayo area, allowing the endorsement of appropriate management measures.

Interestingly, the four dolphins sampled in Cork Harbour were not genetically distinct from the Shannon dolphins, indicating a common origin or gene flow between these areas. Dolphins sampled in Cork Harbour (two males and two females) are part of a small cohesive aggregation of only six individuals that seem to have established in the Cork Harbour area over the past few years (Ryan, Cross & Rogan, 2011). Similar to Cork Harbour, small aggregations that show some degree of site fidelity exist in other coastal areas around the British Isles, such as in the Outer (Grellier & Wilson, 2003) and inner Hebrides (S. Ingram, unpubl. data), and Cornwall (Wood, 1998). However, the origin, level of isolation and long-term viability of such small communities are still poorly understood. Thus, colonization of localized coastal areas by small numbers of individuals is an aspect of bottlenose dolphin biology that requires further investigation, as it may be a key factor in shaping their population structure in coastal waters.

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Supporting Information

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

Figure S1. STRUCTURE runs summary results.

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