

Phylogeography and alpha taxonomy of the common dolphin (*Delphinus* sp.)

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Abstract

The resolution of taxonomic classifications for delphinid cetaceans has been problematic, especially for species in the genera *Delphinus*, *Tursiops* and *Stenella*. The frequent lack of correspondence between morphological and genetic differentiation in these species raises questions about the mechanisms responsible for their evolution. In this study we focus on the genus *Delphinus*, and use molecular markers to address questions about speciation and the evolution of population structure. *Delphinus* species have a worldwide distribution and show a high degree of morphological variation. Two distinct morphotypes, long-beaked and short-beaked, have been considered different species named *D. capensis* and *D. delphis*, respectively. However, genetic differentiation between these two forms has only been demonstrated in the Pacific. We analysed samples from eight different geographical regions, including two morphologically defined long-beaked form populations, and compared these with the eastern North Pacific populations. We found high differentiation among the populations described as long-beaked instead of the expected monophyly, suggesting that these populations may have evolved from independent events converging on the same morphotype. We observed low genetic differentiation among the short-beaked populations across a large geographical scale. We interpret these phylogeographical patterns in the context of life history and population structure in related species.

Introduction

The relationship between morphology and genetic differentiation has been explored in numerous studies (see review by Foote, 1997). Although many comparative studies suggest similar phylogenetic reconstructions using morphological and molecular data (see Patterson *et al.*, 1993), it is clear that the rate and pattern of evolution can differ considerably at neutral and quanti-

tative markers (e.g. Rocha-Olivares *et al.*, 2001). The rate of morphological change can be very rapid in both plants and animals, especially under artificial selection (see review by Roopnarine, 2003). Therefore, conspecific populations have the potential to adapt to local habitats over a timeframe that is short enough to not be reflected in patterns of neutral genetic differentiation accumulated by drift. At the same time, populations not under strong selection for morphological characters, and geographically isolated, may be expected to show greater correspondence between morphological and genetic differentiation. In this study we explore this question with an investigation of a genus within a group of taxa

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that tend to be highly polytypic across their geographical ranges: the delphinid cetaceans.

The evolutionary radiation of the family Delphinidae (oceanic dolphins; see Rice, 1998) is likely to date back to the Middle Miocene, though much of the speciation in this group probably dates to the Pliocene (e.g. Barnes, 1976). A previous phylogenetic study based on the mtDNA cytochrome *b* locus proposed division into five subfamilies, but these have yet to be confirmed (LeDuc *et al.*, 1999). This same study suggested polyphyly within the Delphininae (a subfamily designation that has been supported by various authors; Rice, 1998). This was especially the case among the genera *Delphinus*, *Tursiops* and *Stenella*. Both *Delphinus* and *Tursiops* have been historically divided (based on morphology) into multiple species, though modern classifications had indicated just one species in each genus (see Rice, 1998) until recent genetic studies indicated subdivision into at least two species for each genus. For *Tursiops* we recently proposed a third species based on genetic analyses of a broader geographical range of specimens (Natoli *et al.*, 2004).

In this study we focus on the genus *Delphinus*. *Delphinus* species are widely distributed in all oceans, from temperate to tropical waters and show high mobility across their habitat. Morphological diversity had led to more than 20 different species being described in the past, although they were all subsequently considered local variations of a single species *Delphinus delphis* (Hershkovitz, 1966). The present classification within this genus is still uncertain, although two different species are generally accepted: a long-beaked form (*Delphinus capensis*) and a short-beaked form (*D. delphis*). Our objective in this study is to use the example of this genus to explore the relationship between morphotypic differentiation, habitat use, demographic history and genetic differentiation, to gain insight into the mechanisms that underlie population differentiation and speciation in these marine taxa.

The separation between the long- and short-beaked forms is based on both external morphological characters, such as the colour pattern and overall body size, and skeletal morphological characters, including the length of the rostrum, the tooth and the vertebra counts (Heyning & Perrin, 1994). Specific status for these two forms was based largely on the morphological and genetic analysis of two sympatric populations occurring along the coast of California. In this area, the bigger long-beaked form prefers shallow coastal waters, whereas the smaller short-beaked form occurs mainly in deeper oceanic waters. From the analysis of mtDNA control region sequences, Rosel *et al.* (1994) found reciprocal monophyly, genetic divergence of 1.11%, and fixed differences. Comparison between short-beaked individuals from southern California and short-beaked individuals from other oceans (eastern tropical Pacific and the Black sea) differed by only 0.02% (Rosel *et al.*, 1994).

However, the classification *D. delphis* vs. *D. capensis* remains controversial. High variability of the rostral length-zygomatic width ratio is observed both in the North Pacific and North Atlantic (see Bell *et al.*, 2002; Murphy, 2004). The South African long-beaked form, although very similar to the Pacific long-beaked form in coloration, rostral length, and tooth count, differs for the average total vertebra count (Heyning & Perrin, 1994). In the Indo-Pacific (from the Middle East to China) an extreme long-beaked form has been observed with evidence of clinal variation in the size as one moves east or west from India. This form has been named *Delphinus delphis tropicalis* by Van Bree (1971), though it has also been suggested that it could be a subspecies of *D. capensis* (Jefferson & van Waerebeek, 2002). Possible paraphyly of the Indian and Pacific long-beaked form has also been suggested by LeDuc *et al.* (1999) and reinforced by Jefferson & Waerebeek (2002), suggesting differentiation between the eastern Pacific long beaked common dolphin and the Indo-Pacific *D. capensis*.

Most data suggest different geographical distributions for the two forms and few areas where they overlap (Perrin, 2002). The short-beaked common dolphin shows a continuous distribution north-south along both the eastern and the western coasts of the Atlantic Ocean, and the eastern coast of the Pacific Ocean. It is also present from central Japan to Taiwan, around New Caledonia, New Zealand and Tasmania in the western Pacific, and in the Mediterranean and Black Seas. Conversely the long-beaked form has a disjunct distribution along the western coast of Africa, South Africa and Madagascar, along the eastern coast of South America (from Venezuela to Argentina), from southern California to central Mexico in the eastern Pacific, around Korea, southern Japan and Taiwan in the western Pacific. The form *D. d. tropicalis* is observed only in the northern Indian Ocean and Southern Asia (Perrin, 2002).

Our earlier results suggest possible mechanisms for the evolution of population structure and speciation in highly mobile and social marine organisms. This has been based on comparing population structure among morphotypes and species with contrasting behaviours (e.g. for the closely related *Tursiops truncatus*; Natoli *et al.*, 2004, 2005). In this study we assessed the molecular phylogenetics of recognized morphotypes, and tested the hypothesis that the long-beaked and short-beaked forms represent separate species throughout their distributional range. Reciprocal monophyly would have suggested early divergence and subsequent expansion into their modern distribution. However our data instead suggest that the long-beaked form originated independently in different regions, which implies that selection for this morphotype represents adaptation to local environments, and may be driving local speciation.



Fig. 1 Map of sample locations. Abbreviations are as in Table 1.

Materials and methods

Sample collection and DNA extraction

In total, 199 *Delphinus* sp. samples from eight geographical regions were analysed in this study (Fig. 1). Samples from the eastern North Atlantic (Galicia, Celtic Sea and Scotland), the eastern central Atlantic (Açores, Canary Islands and Madeira) and western North Atlantic were described as short-beaked form (*D. delphis*). Samples from South Africa were described as long-beaked form (*D. capensis*). Samples from Mauritania were described as long-beaked form, although the comparison of the skull measurements with the published data in Heyning & Perrin (1994) classified the individuals as follows: MAU1, very likely short-beaked; MAU2 clearly short-beaked; MAU3 and MAU4, likely long-beaked; MAU5, clearly long-beaked, MAU6, unclear (A. Aguilar, unpublished data). Samples from Argentina were labelled *D. delphis*, although the actual form was not determined (Table 1). Samples were obtained from stranded dolphins or dolphins accidentally caught in nets. Samples from Mauritania and two samples from the Açores were bone specimens.

DNA was extracted from tissue samples preserved in salt saturated 20% DMSO by a standard phenol/chloroform extraction method (Hoelzel, 1998). DNA was extracted from bone samples using QIAgen polymerase chain reaction (PCR) purification columns after grinding 100 mg of bone and digesting it at 37 °C for 48 h in 1 mL of digestion buffer (0.01 M Tris, 0.01 M NaCl, 1% SDS, 2 mg mL⁻¹ proteinase K, 0.01 PTB). The extraction and the analysis of the bone specimens were conducted in a different laboratory where no cetacean DNA had ever been manipulated before, to avoid contamination. An

Table 1 List of the populations analysed and correspondent acronyms.

Population	Acronym	Microsatellite	mtDNA
Galicia, Gulf of Biscay	GAL	39	36
Celtic	CEL	41	29
Scotland	SCO	26	21
Eastern Central Atlantic	ECA	13	14a
Western North Atlantic	WNA	13	11
Mauritania	MAU	–	6
Argentina	ARG	18	15
Short-beaked Pacific	sbPA	–	13b
Long-beaked Pacific	lbPA	–	11b
Long-beaked South Africa	lbSA	43	20

The number of samples for each population considered in this article are reported for the microsatellite and the mtDNA analyses. Data taken from other publications are as follows: (a) four sequences from Açores were from J. Matzen Silva, R. Norberto, J. Matos, D. Mendonca, F. Simoes & J. Azevedo, unpublished data, (b) from Rosel *et al.* (1994).

extraction including everything but tissue was carried through all the analyses as a negative control.

Sex determination

Individuals whose gender was unknown were sexed by amplifying portions of the genes ZFX and ZFY as described in Bérubé & Palsbøll (1996).

Microsatellite analysis

Nine published microsatellite loci were analysed. Primers KWM1b, KWM2a, KWM2b, KWM9b, KWM12a were

derived from *Orcinus orca* (Hoelzel *et al.*, 1998), EV37Mn from *Megaptera novaeangliae* (Valsecchi & Amos, 1996), TexVet5, TexVet7 and D08 from *T. truncatus* (Shinohara *et al.*, 1997; Rooney *et al.*, 1999). Microsatellites were not successfully amplified on the Mauritania samples, due to the highly degraded status of the DNA extracted from these samples and the relatively large size of most of the microsatellite markers. Amplified DNA was analysed for length variation on 6% polyacrylamide denaturing gels using fluorescent imaging on an automated ABI PRISM 377 DNA sequencer, after incorporation of 1/10 fluorescent labelled primer (PCR reaction conditions: 100 μ M dNTPs, 0.75–1.5 mM MgCl₂, 10 mM Tris-HCl pH 8.4, 50 mM KCl, 200 nM of each primer, 0.02 U μ L⁻¹ Taq polymerase. PCR cycling profile: 5 min at 95 °C; then 35 cycles of 40 s at 94 °C, 1 min at the T[°]ann, 1 min at 72 °C; then 10 min at 72 °C). The annealing temperatures (T[°]ann) were as follows: KWM1b: 48 °C; KWM2a: 48 °C; KWM2b: 44 °C; KWM9b: 62 °C; KWM12a: 56 °C; EV37Mn: 52 °C; TexVet5: 49 °C; TexVet7: 49 °C; D08: 57 °C. An internal standard marker (Genescan-500 ROX, Applied Biosystems, Warrington, UK) was used to determine the allele sizes.

For microsatellite loci, the level of polymorphism was estimated as the number of alleles per locus, observed heterozygosity (H_o), expected heterozygosity (H_e) and allelic richness. Allelic richness controls for variation in sample size by a rarefaction method, and was calculated using the program FSTAT 2.9.3 (Goudet, 2001). Evaluation of possible deviations from the expected Hardy–Weinberg (HW) equilibrium (overall deviation, heterozygote deficiency and heterozygote excess) were performed using Fisher's exact test and the Markov chain method (dememorization number, number of batches, iteration per batch set at 1000, Bonferroni correction applied).

Genetic differentiation among populations was assessed based on both the infinite allele model using F_{ST} , and the stepwise mutation model using Rho_{ST} . The level of differentiation between population pairs was estimated as F_{ST} (Weir & Cockerham, 1984) using the program ARLEQUIN 2.0 (Schneider *et al.*, 1999) and Rho_{ST} using the program RSTCALC (Goodman, 1997). The significance of the difference of F_{ST} and Rho_{ST} values from zero was tested by permutation analysis. A permutation test to assess differentiation for allele size was performed for F_{ST} and Rho_{ST} using the program SPAGED1 (Hardy & Vekemans, 2002).

The most probable number of putative populations (K) that best explains the pattern of genetic variability was estimated using the program STRUCTURE 2.1 (Pritchard *et al.*, 2000). We assumed the admixture model and performed the analysis considering both the independent and the correlated allele frequency model. Burn in length and length of the simulation was set at 100 000 and 1 000 000 repetitions, respectively. To test the convergence of the priors and the appropriateness of the chosen burn in and simulation lengths, we ran a series of

independent runs for each value of K (for $1 \leq K \leq 7$) as suggested by Pritchard *et al.* (2000). We tested whether any particular individual was an immigrant or had an immigrant ancestor, by using the model with prior population information, subdividing the individuals into K populations, according to the results of the previous analysis. We assumed ν (migration rate) = 0.05 and 0.1, and testing for $0 \leq$ number of generations (G) ≥ 2 .

Sex-biased dispersal was tested using the program FSTAT 2.9.3 based on sex-specific expectations with respect to F_{IS} , F_{ST} and a likelihood assignment index (see Goudet, 2001). Only adult individuals (a total of 187: 73 females and 114 males) were considered for this analysis.

An asymmetric estimate of the migration rate ($M = 4N_e m$) between pairwise populations, based on microsatellite and mtDNA data, was calculated using MIGRATE (Beerli, 1997–2002). The length of the runs was optimized for both markers (acceptance–rejection $>2\%$, $R < 1.2$). Initial runs were set estimating θ and M with F_{ST} and allowing M to be asymmetric. Reruns were set using the parameter estimated found with the first run and lengthening the MCMC chains. In order to verify the result a final run was set using longer chains. For comparison the migration rate was also calculated according to $F_{ST} = 1/(4Nm + 1)$.

mtDNA analysis

The first 369 bp at the 5' end of the mtDNA control region were sequenced in a total of 148 samples, whereas other sequences were obtained from the published databases (Table 1). In total 176 sequences of *Delphinus* sp. were available.

The mitochondrial DNA control region was amplified either with universal primers MTCRf (5'-TTC CCC GGT GTA AAC C) and MTCRr (5'-ATT TTC AGT GTC TTG CTT T) after Hoelzel (1998), or with the primers 5'-ACA CCA GTC TTG TAA ACC-3' and 5' -TAC CAA ATG TAT GAA ACC TCA G-3' after Rosel *et al.* (1994).

The PCR reaction conditions were as follows: 100 μ M dNTPs, 1.5 mM MgCl₂, 10 mM Tris-HCl pH 8.4, 50 mM KCl, 200 nM of each primer, 0.02 U μ L⁻¹ Taq polymerase. The PCR cycling profile was 4 min at 95 °C, 35 cycles of 45 s at 94 °C, 1.5 min at 50 °C, and 1.5 min at 72 °C, followed by 8 min at 72 °C. PCR products were purified with QIAGEN PCR purification columns and sequenced directly using the ABI dye-terminator method.

Mitochondrial DNA from the Mauritania samples was amplified using two sets of primers designed in order to amplify two overlapping portions of the control region of approximately 200 bp each (Dmtcrf: 5'-TTA GTC TCT CCT TGT AAA T-3' and Dmtcrr: 5'-GGT GAT TAA GCT CGT GAT-3'; Nichols, 2004. MTCRf and mtancr: 5'-AAA ATA AAT GAA TGC ACA ATA-3', designed by the author). The PCR reaction conditions were as follows: 100 μ M dNTPs, 2.5 mM MgCl₂, 10 mM Tris-HCl pH 8.4,

50 mM KCl, 200 nM of each primer, 0.4 $\mu\text{g } \mu\text{L}^{-1}$ BSA, 0.02 U μL^{-1} Taq polymerase. The PCR cycling profile was 15 min at 95 °C, 45 cycles of 45 s at 94 °C, 1.5 min at 47 °C and 1.5 min at 72 °C, followed by 8 min at 72 °C.

Sequences were compared with 25 published sequences from two populations of common dolphins from the Pacific Ocean described as short-beaked and long-beaked form, respectively (Rosel *et al.*, 1994). Another four published sequences from Açores were also included in the analysis (Matzen Silva *et al.*, 2002).

Sequence alignment was performed using ClustalX (Thompson *et al.*, 1997). The degree of differentiation (F_{ST} and Φ_{ST}) and Tajima's D were estimated using ARLEQUIN 2.0 (Schneider *et al.*, 1999). Estimates of Φ_{ST} used the Tamura-Nei genetic distance model (Tamura & Nei, 1993).

Genetic distance (Da) was estimated using Tamura-Nei with the SENDBS programme, written by N. Takezaki (National Institute of Genetics, Mishima, Shizuoka, Japan; <http://www.cib.nig.ac.jp/dda/ntakezak-j.html>). SENDBS was also used to estimate π .

Individual haplotypes were compared by the Neighbour-Joining method using PAUP* 4.0B10 (Swofford, 1997) and rooted with homologous sequence from *Stenella attenuata*. Majority-rule consensus trees were constructed from 1000 bootstrap replications and a 50% criterion for the retention of nodes was applied. Distances were based on Tamura-Nei as above. The *ti/tv* ratio was set at 6.5, based on observed values. A phylogenetic reconstruction based on a Bayesian approach was also implemented using the programme MRBAYES 3.0B4 (<http://mrbayes.csit.fsu.edu/index.php>). We used the general time reversible rate model, with gamma distributed rate variation across sites. The number of generations for the MCMC method was set to 500 000 and a tree was saved every 100 generations. The consensus tree was produced using PAUP* 4.0B10 (Swofford, 1997) retaining branches with 50% support or greater. A median-joining network was generated to infer phylogenetic relationships among the mtDNA haplotypes using the program NETWORK 4001 (Bandelt *et al.*, 1999; <http://www.fluxus-engineering.com/fluxe02.htm>).

Results

Microsatellite analysis

Population differentiation

Expected (H_e) and observed (H_o) heterozygosities were calculated for each population at each locus (Table 2). Loci that significantly deviated from the HW equilibrium were found in all populations with the exception of ARG. In all cases deviation from HW equilibrium was due to significant heterozygosity deficiency (P -values <0.05). The locus TexVet5 significantly deviated from the HW

equilibrium in the largest number of populations. Omission of this locus did not change the pattern of differentiation among populations (F_{ST} and RHO_{ST} tests; data not shown), so data are presented based on all loci. The locus KWM1b was monomorphic in the lbSA population. The lbSA population showed the lowest average observed and expected heterozygosities and the lowest allelic diversity. The other populations showed similar values for both the average heterozygosities and allelic diversity parameters.

Genetic differentiation among pairwise populations was estimated using F_{ST} and Rho_{ST} (Table 3). The lbSA population showed the highest differentiation compared to all the other populations, with both F_{ST} and Rho_{ST} analyses. The ARG population was also significantly differentiated from all the other populations based on F_{ST} . The eastern Atlantic populations (GAL, CEL, SCO and ECA) clustered together showing low or no genetic differentiation. The WNA population was significantly differentiated from all populations except SCO for F_{ST} .

We tested the role of allele size in determining population differentiation comparing F_{ST} and Rho_{ST} values (after Hardy & Vekemans, 2002). This suggested that allele size does not contribute to the differentiation of the populations we analysed, and therefore F_{ST} should be preferred to Rho_{ST} .

Bayesian analysis was used as an alternative approach to test for population structure. The number of populations (K) that best explained the genetic variability observed across our samples was found to be three (the estimated ln probability of K for $K = 2$ was -6497.2; for $K = 3$ was -6475.8; and for $K = 4$ was -6587.1; posterior probability of $K = 3$ is 1.00). Given the lack of conformation to HW expectations for one locus (TexVet5) in several populations, we repeated the analysis omitting this locus, and $K = 3$ retained the highest likelihood ($K = 2$: -5704.7; $K = 3$: -5699.7; $K = 4$: -5899.7; posterior probability of $K = 3$ is 0.98). Consistency among different runs was observed for the estimate of $P(X/K)$ and the prior α , indicating that the burn-in length and the length of the runs were appropriate. The same result was found using both correlated allele frequency and independent allele frequency models. The Bayesian analysis identified three main clusters of populations (Fig. 2). The lbSA population highly differentiated from all the others, a western Atlantic cluster (including WNA and ARG), and an eastern Atlantic cluster (including GAL, CEL, SCO, ECA).

Migrants and sex-biased dispersal

We analysed whether individuals were possible immigrants or descendants of recent immigrants considering the three populations identified by the Bayesian analysis. Three possible immigrant individuals were identified. All individuals were migrants from the second cluster (WNA, ARG) to the first cluster (GAL, CEL, SCO). Two individuals were from GAL and they were identified as possible

Table 2 Number of alleles (number of private alleles in parentheses, allelic richness in square brackets), expected (H_e) and observed (H_o) heterozygosities for each population at each microsatellite locus.

	Populations						
	GAL, 39	CEL, 41	SCO, 26	ECA, 13	WNA, 13	ARG, 18	lbSA, 43
KWM1b							
N. Alleles 4	4 [2.6]	3 [2.66]	3 [2.18]	4 [3.92]	2 [2]	2 [1.94]	1 [1]
H_o	0.21	0.219	0.115	0.308	0*	0.125	0
H_e	0.271	0.246	0.149	0.508	0.221	0.179	0
KWM2a							
N. Alleles 18	13 [10.43]	13 [8.57]	12 (1) [9.58]	11 [10.54]	8 [7.92]	11 [9.76]	9 [7.81]
H_o	0.846	0.878	0.76	0.769	0.615*	0.889	0.93
H_e	0.901	0.844	0.893	0.895	0.895	0.913	0.851
KWM2b							
N. Alleles 8	7 [6.43]	6 [5.85]	6 [5.92]	6 [5.99]	6 [6]	5 [4.94]	5 [4.2]
H_o	0.795	0.878	0.808	0.769	0.667	0.667	0.619
H_e	0.825	0.824	0.827	0.84	0.837	0.756	0.61
KWM9b							
N. Alleles 16	13 (1) [9.59]	11 [8.8]	9 [7.96]	10 [9.76]	10 (1) [10]	12 [10.65]	8 [6.1]
H_o	0.897	0.927	0.769	0.923	0.917	0.937	0.805
H_e	0.892	0.869	0.874	0.917	0.888	0.897	0.78
KWM12a							
N. Alleles 14	9 [7.47]	10 [7.31]	9 [7.16]	8 (1) [7.77]	11 (1) [10.61]	8 [6.96]	8 [6.57]
H_o	0.795	0.78	0.808	0.692	0.923	0.722	0.683
H_e	0.791	0.805	0.771	0.831	0.905	0.825	0.815
EV37Mn							
N. Alleles 31	20 [11.84]	18 [11.54]	19 (2) [12.94]	14 (1) [13.3]	12 [11.6]	11 [9.65]	12 (1) [7.11]
H_o	0.949	0.805	0.885*	0.846	0.923	0.823	0.721
H_e	0.918	0.903	0.912	0.898	0.914	0.895	0.814
TexVet5							
N. Alleles 14	10 (1) [7.63]	9 [7.07]	10 [9.14]	9 [9]	8 [8]	10 (1) [8.8]	8 [6.83]
H_o	0.553**	0.575*	0.461**	0.583*	0.667	0.667	0.762*
H_e	0.846	0.803	0.903	0.931	0.851	0.88	0.84
TexVet7							
N. Alleles 8	5 [4.83]	6 [4.49]	5 [4.57]	5 [4.85]	7 (1) [6.92]	7 [6.29]	4 [3.86]
H_o	0.667	0.8	0.538	0.538	0.692	0.571	0.69
H_e	0.746	0.708	0.744	0.68	0.828	0.698	0.654
D08							
N. Alleles 21	16 (2) [9.74]	11 [7.45]	11 [8.61]	8 [8]	12 (1) [11.5]	13 [11.05]	8 (1) [6.04]
H_o	0.667**	0.78	0.8	0.75	0.846	0.778	0.658
H_e	0.863	0.808	0.856	0.855	0.889	0.913	0.716
Average (SD)							
N. Alleles	10.78 (5.2)	9.7 (4.4)	9.3 (4.7)	8.3 (3.1)	8.4 (3.2)	8.8 (3.6)	7 (3.2)
	[7.84 (2.9)]	[7.08 (2.6)]	[7.56 (3.1)]	[8.13 (2.9)]	[8.28 (3.1)]	[7.78 (3)]	[5.5 (2.1)]
H_o	0.71 (0.22)	0.74 (0.22)	0.66 (0.25)	0.69 (0.18)	0.69 (0.29)	0.69 (0.24)	0.65 (0.26)
H_e	0.78 (0.2)	0.76 (0.2)	0.77 (0.24)	0.82 (0.14)	0.80 (0.22)	0.77 (0.23)	0.68 (0.27)

Allelic richness was calculated on a minimum number of samples of 12 individuals.

Abbreviations are as in the caption of Table 1.

*Loci with a P -value <0.05 when tested for heterozygote deficiency.

**Loci still significant after Bonferroni correction (P -value <0.00079).

immigrants at both the values of $\nu = 0.01$ and $\nu = 0.05$ ($P = 0.001$). One individual was from SCO and was identified as an immigrant only for $\nu = 0.05$ ($P = 0.01$). All individuals had higher probabilities to be immigrants than having immigrant ancestry.

Although the Bayesian analysis clustered the WNA and the ARG populations in the same group, the F_{ST}

values indicated significant differentiation between these two populations. Therefore, we calculated the migration rate (M) considering four main populations: the EA (eastern Atlantic) population (including GAL, CEL, SCO and ECA), the WNA population, the ARG population, and the lbSA population. The results are reported in Table 4. Sex-biased dispersal among regions was tested

Table 3 Genetic differentiation among pairwise populations using microsatellite data.

	<i>n</i>	GAL	CEL	SCO	ECA	WNA	ARG	lbSA
GAL	39		0.008	0.03**	0.008	0.02	0.049**	0.164***
CEL	41	0.005		0.011	0.004	0.028*	0.088***	0.201***
SCO	26	0.012*	0.011**		0.028	0.025	0.061**	0.171***
ECA	13	-0.002	0.001	0.01		-0.019	0.032	0.12***
WNA	13	0.014*	0.023***	0.012	0.021*		-0.002	0.095***
ARG	18	0.037***	0.045***	0.034***	0.041***	0.037***		0.1***
lbSA	43	0.1***	0.105***	0.103***	0.099***	0.087***	0.076***	

F_{ST} values are reported below the diagonal, whereas Rho_{ST} values are reported above the diagonal.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

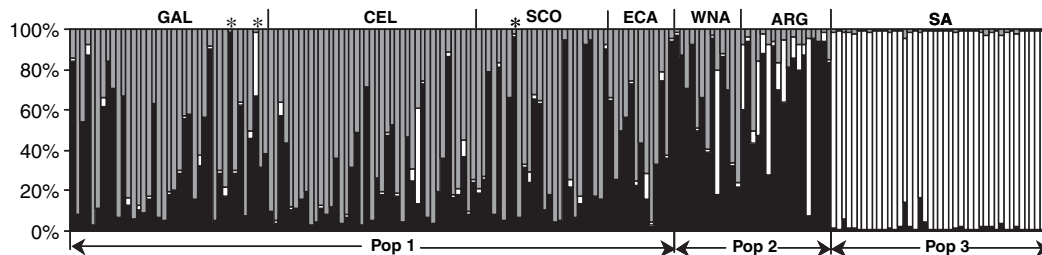


Fig. 2 Estimated proportion of the coefficient of admixture of each individual's genome that originated from population k , for $K = 3$ (below the graphic). Each individual is represented by a column. Geographical origin of the samples is reported above the graphic. Abbreviations are as in Table 1. The asterisks indicate the individuals identified as migrants.

using F_{STAT} (see Methods), and no significant pattern was found to indicate a greater propensity for dispersal for either sex.

Mitochondrial DNA analysis

Population diversity and differentiation

Mitochondrial control region sequences from the seven populations analysed above were compared with sequences from Mauritania (MAU) and with published sequences from two populations in the Pacific Ocean identified as long-beaked form (lbPA) and short-beaked form (sbPA).

Among the 176 sequences analysed, 96 haplotypes were identified, showing 76 polymorphic sites.

Shared haplotypes were common among the eastern Atlantic populations (GAL, CEL, SCO, ECA) and the WNA population. MAU, ARG and the Pacific Ocean populations (lbPA, sbPA) did not show any shared haplotypes with other populations. The lbSA population shared one haplotype with the GAL population.

Average gene and nucleotide diversities were estimated for each population (Table 5). Diversities were relatively high for all the populations analysed. The lbSA population showed the lowest gene diversity, whereas the lbPA population showed the lowest nucleotide diversity. Neutrality tests were performed. Although Tajima's D -values were not statistically significant, Fu's

F_s values were large, negative and significant for the GAL, ECA, lbPA and sbPA populations, suggesting possible population expansion (Table 5).

Genetic differentiation among pairwise populations was estimated using F_{ST} and Φ_{ST} (Table 6). The F_{ST} values confirmed the population structure suggested by the nuclear markers ($r_6 = 0.876$, $P = 0.008$). Three populations from the eastern Atlantic (GAL, CEL & SCO) showed no significant differentiation, whereas lbPA was differentiated from all other populations except ARG. The lbSA and lbPA populations, both classified as the long-beaked form, were highly differentiated.

No significant correlation was found between the F_{ST} and Φ_{ST} matrices ($r_9 = -0.004$, $P = 0.38$). The Φ_{ST} values suggested a different scenario indicating no significant differentiation between the WNA population and two eastern Atlantic populations (ECA and SCO), or the ARG population. Conversely, the MAU population was highly differentiated from all other populations based on Φ_{ST} , including the two long-beaked populations (lbPA and lbSA), but not for F_{ST} . No significant differentiation was observed between the sbPA and the CEL populations. The lbSA did not show significant differentiation when compared with the WNA and ARG populations, whereas it was highly differentiated from the lbPA population.

The migration rate (M) based on mtDNA sequences was calculated for the same four populations considered for the microsatellite analysis. The results are reported

Population	Microsatellites (bi-parental)				mtDNA (maternally inherited)			
	<i>Nm</i>	1,2	2,1	95% CI	<i>Nm</i>	1,2	2,1	95% CI
1 EA	17.08	–	5.21	4.83–5.62	10.04	–	20.82	9.37–35.63
2 WNA		9.36	–	8.68–10.09		1.510	–	0.26–4.00
1 EA	6.90	–	7.09	6.64–7.56	14.03	–	5.64	3.11–13.36
2 ARG		10.69	–	9.99–11.44		0.000	–	0.00–0.56
1 EA	2.41	–	4.23	3.88–4.6	5.29	–	5.69	3.27–11.06
2 SA		3.90	–	3.54–4.25		0.316	–	0.24–1.09
1 WNA	6.56	–	13.40	12.46–14.41	8.02	–	0.04	0.03–0.82
2 ARG		12.94	–	12.11–13.8		9.180	–	3.34–30.29
1 WNA	2.62	–	6.19	5.48–6.88	3.62	–	0.00	0.00–0.395
2 SA		2.53	–	2.26–2.82		1.600	–	0.67–3.03
1 ARG	3.03	–	6.84	6.14–7.63	5.07	–	43.90	22.70–70.85
2 SA		2.71	–	2.42–3.11		0.740	–	0.67–2.70

Nm columns refer to the values calculated according to $F_{ST} = 1/(4Nm + 1)$. The other columns refer to the asymmetrical migration rate calculated using a maximum likelihood method (MIGRATE): 1,2 stands for: migration from population 1 to population 2; 2,1 stands for: migration from population 2 to population 1. The confidence interval (95% CI) is also reported. Abbreviations for the populations are as in Table 1 except for EA that stands for eastern Atlantic populations (GAL, CEL, SCO, ECA).

Table 5 Gene diversity, nucleotide diversity, Tajima's *D* and Fu's *F_s* values are reported for each population.

Pop	N. seq	gene div.	nucl. div.	<i>D</i>	<i>F_s</i>
GAL	36	0.967	0.017	–0.825	–13.4***
CEL	29	0.958	0.018	–0.446	–5.6*
SCO	21	0.943	0.015	–0.697	–3.03
ECA	14	0.989	0.018	–0.533	–6.4**
WNA	11	0.909	0.013	–0.434	–0.717
MAU	7	0.952	0.019	–0.249	–0.7
ARG	15	0.971	0.019	–0.434	–3.53*
sbPA	13	1	0.021	–1.183	–8.27***
lbPA	11	0.982	0.012	–0.719	–5.15**
lbSA	20	0.853	0.0169	–0.124	1.1

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

in Table 4. Estimates using mtDNA suggest some directional bias in dispersal, especially between WNA and EA, and between ARG and SA. Our theta estimates from MIGRATE ranged from 0.012 to 0.155 for the mtDNA data. Simulation studies showed that theta of 0.025 gave good estimates of migration rates under similar conditions (see Table 3 in Abdo *et al.* 2004).

Rooted (*S. attenuata*) Neighbour-Joining and Bayesian trees were reconstructed using all 96 haplotypes (Fig. 3). Both methods showed that the lbPA population diverged from the other haplotypes, but was not monophyletic with the lbSA population. The Neighbour-Joining tree resolved fewer lineages than the Bayesian tree. The latter included highly supported lineages, but none of these apart from lbPA reflected any clear geographic clustering.

Table 4 Estimate of the migration rate (*M*) between populations based on the microsatellite and mtDNA data.

Discussion

Our results showed significant genetic differentiation among populations inhabiting different oceans (Indian vs. Atlantic), and different sides of the same ocean (eastern Atlantic vs. western Atlantic) but little or no differentiation among populations inhabiting the same side of an ocean basin (Tables 3 and 6). The Bayesian analysis based on individual genotypes (implemented in STRUCTURE), suggested similar population partitioning, identifying three main clusters corresponding to the lbSA population (Indian Ocean), the WNA/ARG population (western Atlantic Ocean) and the other populations (eastern Atlantic Ocean; Fig. 2). The lbSA population was the most differentiated, reflecting the difference in morphology between the long-beaked and short-beaked forms.

However, mtDNA phylogenetic analyses did not support the hypothesis of one single long-beaked lineage worldwide. Among phylogenetic clusters, the only one that reflected local geographical origin was the lbPA population lineage. No shared haplotypes or fixed mutations were observed among the populations described as long-beaked (lbSA, lbPA and MAU). F_{ST} and Φ_{ST} values suggested high divergence between the lbPA and lbSA populations, higher than between the lbSA and the short-beaked populations (Table 6), and suggested that the lbPA and lbSA populations had independent origins within the broader lineage. Both the lbPA and MAU 'long-beaked' populations showed higher Φ_{ST} than F_{ST} values for all pairwise comparisons with other populations. This is expected when similar haplotypes are associated geographically, and the mutation process has been more important relative to other causes of genetic differentiation. Conversely, the lbSA population showed

Table 6 Genetic differentiation among pairwise populations using mtDNA data.

	<i>n</i>	GAL	CEL	SCO	ECA	WNA	MAU	ARG	sbPA	lbPA	lbSA
GAL	36		0.005	0.0001	0.077**	0.059*	0.16**	0.132***	0.051**	0.465***	0.099**
CEL	29	0.003		-0.009	0.044*	0.015	0.13*	0.099**	0.032	0.443***	0.058*
SCO	21	-0.011	0.007		0.012	0.037	0.204***	0.124***	0.053*	0.481***	0.083**
ECA	14	-0.003	-0.01	-0.006		0.078*	0.244***	0.127***	0.103***	0.466***	0.104**
WNA	11	0.045**	0.05**	0.053*	0.044*		0.208***	0.029	0.045*	0.54***	0.065
MAU	6	0.019	0.024	0.033	0.006	0.05		0.227***	0.1**	0.5***	0.193*
ARG	15	0.031**	0.036***	0.043***	0.02*	0.059***	0.0159		0.105***	0.506***	0.026
sbPA	14	0.018*	0.022*	0.029*	0.006***	0.044***	0.0	0.014		0.412***	0.107**
lbPA	11	0.026*	0.031*	0.039*	0.015*	0.055***	0.009	0.024	0.009***		0.521***
lbSA	20	0.083***	0.093***	0.102***	0.082***	0.121**	0.086	0.09***	0.076**	0.087**	

F_{ST} values are reported below the diagonal whereas Φ_{ST} values are reported above the diagonal.

* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

similar Φ_{ST} and F_{ST} values, suggesting recent population divergence. Recent divergence of the lbSA population is also supported by the microsatellite DNA analysis. The lack of significance for microsatellite allele size in determining population differentiation suggests that genetic drift is important and the rate of gene flow may be high relative to the mutation rate.

Among the short-beaked form populations, STRUCTURE identified two clusters dividing the eastern Atlantic populations (GAL, CEL, SCO, ECA) from the western Atlantic populations (WNA, ARG). However, in the eastern Atlantic, nuclear and mtDNA data showed little indication of differentiation among populations. The low or nonsignificant F_{ST} values (Tables 3 and 6) and the high number of shared haplotypes suggest high gene flow among these regions, despite the large geographical distances. Low F_{ST} values also suggest possible gene flow across the Atlantic Ocean, between the WNA and the eastern Atlantic populations. Both MIGRATE and Nm estimated from F_{ST} indicated relatively high rates of gene flow between the eastern and western North Atlantic.

The mtDNA data from MIGRATE suggests a possible bias in the long-term direction of migration for females, especially from west to east in the North Atlantic (consistent with the data from STRUCTURE), and from east to west in the South Atlantic. It also suggested a tendency for females to emigrate from South Africa and the western North Atlantic, and immigrate to Argentina and the eastern North Atlantic. The North Atlantic data could be consistent with oceanic current patterns, but there are no data for any causal relationships. In marine mammals, population structure is generally more evident for mtDNA than nuclear markers, in part because of the different effective population size represented by the two genomes, but likely also because of the more frequent dispersal of males (e.g. Hoelzel *et al.*, 2002). In this case, we found no evidence for a strong sex-bias with respect to overall dispersal rate.

The structure of *D. delphis* populations was in contrast to that seen for the closely related *Tursiops* species. The

bottlenose dolphin (genus *Tursiops*) shows a high degree of morphological variation, and strong genetic differentiation has been reported even between parapatric populations (Mead & Potter, 1995, Hoelzel *et al.*, 1998). Different species have recently been recognized within this genus: the coastal *aduncus* form in Chinese waters (Wang *et al.*, 1999) and the South African *aduncus* form (Natoli *et al.*, 2004). Some authors (Hoelzel *et al.*, 1998; Natoli *et al.*, 2004, 2005) suggest that specialization for local resources by the consolidation of different feeding strategies among groups might favour philopatry, and lead to progressive genetic divergence.

As *Delphinus* alpha taxonomy defined by beak length and associated morphology was not supported by the genetic data, morphotypic variation may relate more to local adaptation than differentiation along phylogenetic lineages. Similar disjunction between the radiation of morphometric characters related to foraging, and shallow or inconsistent patterns of genetic differentiation have been seen in other taxonomic groups, such as Darwin's finches (Freeland & Boag, 1999).

Even within the short-beaked form there is considerable morphological variability within and among populations, sometimes overlapping the distributions for the long-beaked form (Bell *et al.*, 2002; Murphy, 2004). Those populations identified as short-beaked showed low differentiation even over large geographical ranges. This is in contrast to *T. truncatus* populations (Natoli *et al.*, 2004, 2005), and likely reflects a greater propensity for long-range dispersal. At the same time, some local common dolphin populations with long-beaked morphology, may reflect independent founder events (consistent with evidence for relatively low diversity in the South African and Pacific populations, and possible expansion of the Pacific and Mauritanian long-beaked populations) and adaptation to local environments (perhaps like *Tursiops* sp.). Short-beaked populations showed relatively low levels of differentiation (and evidence for gene flow across oceans), reflecting the high mobility, pelagic habitat and fluid social structure typical of these populations. Therefore the evolution of population

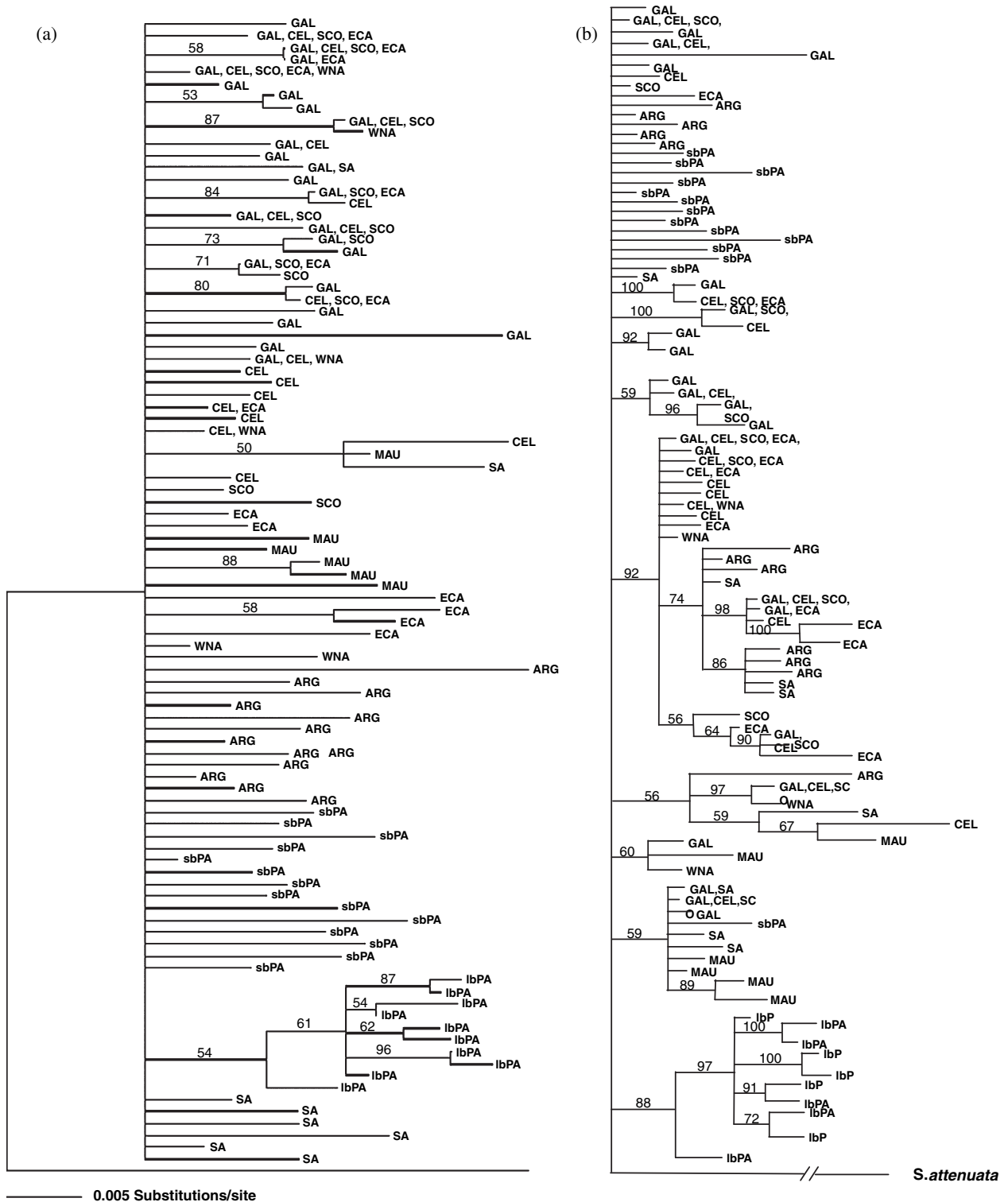


Fig. 3 Neighbour-Joining (a) and Bayesian (b) trees illustrating the phylogenetic relationships among 96 mtDNA haplotypes. Bootstrap and Bayesian values >50% are indicated. *Stenella attenuata* was used as an outgroup.

structure and eventual speciation in these taxa may be based on local founding events and subsequent adaptation of some coastal populations, originating from relatively panmictic oceanic populations.

If selection for beak length and related characters is determining morphology in coastal dolphin populations (both the long-beaked *Delphinus* and *Tursiops* forms inhabit nearshore waters), then this may be related to prey capture in that environment. Beak length tends to be longer in dolphin species that live in relatively shallow, turbid environments (most extreme in the river dolphin species), but we know of no data directly demonstrating this relationship. By this scenario the morphological similarity of long-beaked forms in different parts of the world would be an example of convergence. Convergence almost certainly explains the morphotypic similarity between the genetically highly differentiated 'aduncus-type' bottlenose dolphins off Asia and South Africa (Natoli *et al.*, 2004), and numerous examples of convergence based on feeding morphology have been proposed for marine fish in the family Labridae (Westneat *et al.*, 2005). Further studies should be undertaken to investigate this possibility, together with studies including other polytypic delphinid species (e.g. *Stenella* sp. see Perrin *et al.*, 1981) to test the hypothesis that habitat choice is an important mechanism leading to population structure and speciation in these mobile marine animals.

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