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# Evidence for male dispersal along the coasts but no migration in pelagic waters in dusky dolphins (*Lagenorhynchus obscurus*)

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## Abstract

**Using nine nuclear species-specific microsatellite loci and two mitochondrial gene fragments (cytochrome *b* and control region), we investigated the processes that have shaped the geographical distribution of genetic diversity exhibited by contemporary dusky dolphin (*Lagenorhynchus obscurus*) populations. A total of 221 individuals from four locations (Peru, Argentina, southern Africa, and New Zealand) were assayed, covering most of the species' distribution range. Although our analyses identify a general demographic decline in the Peruvian dusky dolphin stock (recently affected by high natural and human-induced mortality levels), comparison between the different molecular markers hint at an ancient bottleneck that predates recent El Niño oscillations and human exploitation. Moreover, we find evidence of a difference in dispersal behaviour of dusky dolphins along the South American coast and across the Atlantic. While data in Peruvian and Argentine waters are best explained by male-specific gene flow between these two populations, our analyses suggest that dusky dolphins from Argentina and southern Africa recently separated from an ancestral Atlantic population and, since then, diverged without considerable gene flow. The inclusion of a few New Zealand samples further confirms the low levels of genetic differentiation among most dusky dolphin populations. Only the Peruvian dusky dolphin stock is highly differentiated, especially at mitochondrial loci, suggesting that major fluctuations in its population size have led to an increased rate of genetic drift.**

*Keywords*: Cetacea, mtDNA, microsatellites, phylogeography, male-biased gene flow, bottleneck, ancient polymorphism

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## Introduction

The divergence of populations into local or geographical variants within a species is an intuitive starting point for discussing speciation in delphinid cetaceans. Indeed, species of true dolphins often comprise morphotypes that substantially differ in colour pattern, body dimensions, and cranial structure (Perrin *et al.* 1991; Rosel *et al.* 1994; Hoelzel *et al.* 1998b; Wang *et al.* 1999; Jefferson & Van Waerebeek 2002; Kingston & Rosel 2004; Natoli *et al.* 2004). However, species designation in the current taxonomy of cetaceans is clearly problematic (Milinkovitch *et al.* 2002). Groups of individuals are often classified into the same binomial species not because interbreeding or lack of cladistic hierarchy has been demonstrated, but because evidence for separating them into reproductively isolated groups is lacking. Unfortunately, high human-induced mortality levels, especially because of direct and incidental catch in coastal waters, threaten many cetacean populations that might represent such evolutionary distinct lineages or incipient species (Perrin *et al.* 1991; Woodley & Read 1991;

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Van Waerebeek *et al.* 1997; Pichler & Baker 2000). Obviously, one major challenge in the analysis of genetic variation is to work out the relative contributions of distinct processes such as genetic drift, population divergence, gene flow, and selection (all likely to be variable both in time and space). For several reasons, this task is particularly complex in cetaceans. First, many cetacean species show high dispersal abilities and are distributed across habitats where movements are difficult to record and barriers to migration are seldom understood. Second, strongly biased sex-specific dispersal can result in incongruent population histories for males and females (Baker *et al.* 1998; O'Corry-Crowe *et al.* 1997; Bérubé *et al.* 1998; Hoelzel *et al.* 1998b; Brown Gladden *et al.* 1999; Escorza-Trevino & Dizon 2000). Third, complex behaviours such as philopatry and social organization into kinship groups can cause, even in sympatry or on a small geographical scale, significant population subdivision (Hoelzel 1998; Hoelzel *et al.* 1998a). Finally, detailed information on past and recent effective population sizes is lacking in most delphinid species. This often makes data interpretation difficult as fluctuations in population size lead to varying rates of drift, hence, to a nonlinear variation of genetic differentiation through time (Chakraborty & Nei 1977; Hedrick 1999).

In the present paper, we provide insights into the processes shaping geographical patterns of genetic diversity in dusky dolphins. This small delphinid species is distributed in southern hemisphere waters along the coasts of western and eastern South America (Peru, Chile, Argentina), southern Africa (Namibia, South Africa), and New Zealand (Fig. 1a). Although sightings of dusky dolphins have also been recorded from the vicinity of many oceanic island

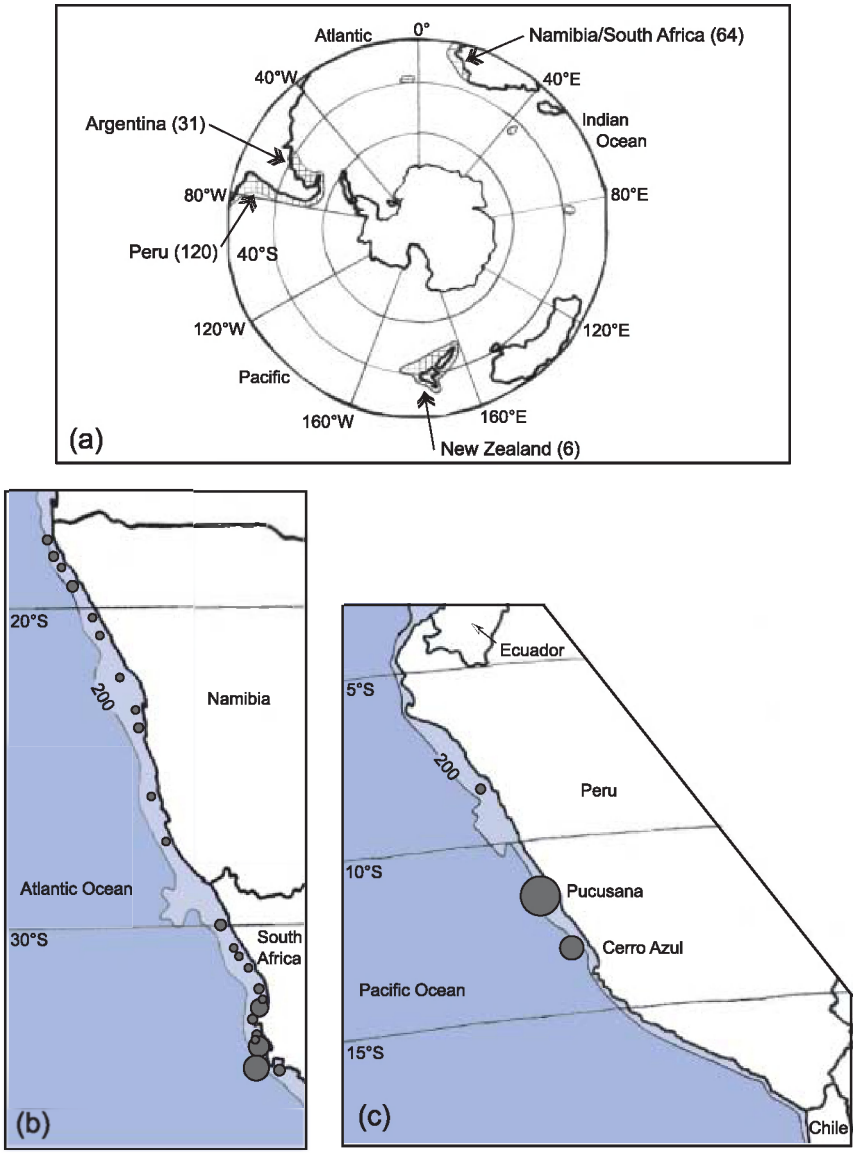


**Fig. 1** (a) Map of the Southern Hemisphere showing general location (arrows) of sampled populations and distribution range of *Lagenorhynchus obscurus* (crosshatched area). Sample sizes are given between parentheses. (b) Sampling sites along the coast of Namibia and South Africa. Tissue samples were collected from stranded or incidentally caught dusky dolphin individuals. Observational data suggest a distribution gap near the Namibian–South African border. (c) Sampling sites in Peruvian waters. They correspond to three harbours where dusky dolphin carcasses are landed after they have been directly caught in local fishery activities. Circle size is proportional to the number of individuals sampled at each site.

groups (e.g. Gough and Falkland Islands in the Atlantic), a continuous pelagic distribution in the southern oceans has not been confirmed (Van Waerebeek *et al.* 1995). Distributional information and morphological studies, which have revealed significant differentiation in cranial characteristics and body size among geographical regions (Van Waerebeek 1993a,b), led authors to propose a disjunct distribution with discrete dusky dolphin stocks confined to continental shelves and islands (Van Waerebeek *et al.* 1995). A comprehensive understanding of the species' population structure is urgently needed given that direct takes of small cetaceans for human consumption and bait in shark fisheries is a major conservation problem, especially in Peruvian waters (Van Waerebeek & Reyes 1990, 1994a,b; Van Waerebeek 1994; Van Waerebeek *et al.* 1997). Furthermore, the upwelling system off Peru and northern Chile forms an extremely rich but very unstable environment. Indeed, recurrent El Niño southern oscillations (ENSO) events with the characteristic warming of surface waters — that blocks the upward mixing of deeper, nutrient-rich water — cause high mortality levels among marine plant, invertebrate, and vertebrate species (Cooper & Laurie 1987; Gilbert *et al.* 2001; Kiesecker *et al.* 2001; Sheppard *et al.* 2002). Preliminary results even show evidence for an R-selection mode of reproduction in Peruvian dusky dolphins, suggesting an adaptation to high losses through an extremely young age at reproduction (Van Waerebeek & Read 1994).

Here, we use multiple markers with different modes of inheritance and different mutation rates to investigate how the closely related, but considerably morphologically differentiated populations evolved. More specifically, we first examined whether a high proportion of genetic diversity has been lost in the Peruvian dusky dolphin stock possibly impacted by recent ENSO and ongoing human exploitation. Second, we investigated population structure and dispersal parameters, such as sex-specific migration rates, and we attempted using, among others, a recently developed Bayesian method (Nielsen & Wakeley 2001; Hey & Nielsen 2004; Hey *et al.* 2004) to distinguish between an equilibrium (ancient population separation with ongoing gene flow) and nonequilibrium (no gene flow, but remnant shared variation as a result of a recent population split) model of population divergence. Third, we examined whether genetic structuring on a small geographical scale could be detected: a distribution gap has been proposed in the proximity of the Namibian–South African border (Fig. 1b) in southern African waters, and dusky dolphins sampled in specific Peruvian harbours might originate from restricted fishing grounds that differ among harbours (Fig. 1c, Van Waerebeek, unpublished data). If clearly characterized, fixed genetic differences among such local groups could provide valuable information to catch statistics studies by allowing assigning individuals of unknown origin to a specific geographical group. Finally, the inclusion of dusky dolphin samples from New Zealand allows us to provide a first insight on how this population is related to other dusky dolphin stocks.

## Materials and methods

### *Tissue collection and DNA extraction*

Skin, bone, and cartilage samples were collected from stranded, incidentally or directly caught dusky dolphin specimens (*Lagenorhynchus obscurus*) covering most of the species' distribution range (Fig. 1a). While exact sampling locations are unknown for the individuals from Argentina ($N = 31$) and New Zealand ($N = 6$), most dusky dolphin samples collected in southern African ($N = 64$) and Peruvian ($N = 120$) waters can be assigned to precise localities along the coast (Fig. 1b,c) possibly allowing for a fine-scale phylogeographical analysis within these populations. For skin samples, standard proteinase K digestion and phenol-chloroform extraction procedures (Hillis *et al.* 1996) were used. Genomic DNA from bone, cartilage, and tooth samples was extracted using the Qiagen DNeasy tissue kit. After a first treatment with emery paper on the outer surface in order to reduce contamination sources, samples were powdered using a mortar either after 2–12 h at −80 °C or with liquid nitrogen. Starting with less than 40 g tissue powder, we followed the Qiagen extraction protocol for animal tissues, but added twice the volume of all solutions in the digestion and precipitation steps and increased incubation time at 55 °C to at least 48 h in a shaking water bath. Only the supernatant was loaded onto the columns in order to avoid any obstructions with nondigested material, and a single elution step was done with 100–150 μL of buffer EB.

### *Sequencing and genotyping*

Two mitochondrial gene fragments (cytochrome *b* and the control region) were amplified and directly sequenced (BigDye Terminator Cycle Sequencing; Applied Biosystems) on both strands on an ABI 377 or ABI 3730 automated sequencer (Applied Biosystems). The full cytochrome *b* gene (1140 bp) was analysed for most samples using two pairs of primers from (Cassens *et al.* 2000). However, probably resulting from degraded DNA, these amplifications failed for most bone, cartilage, and tooth samples, and we designed three new primer pairs, defining smaller fragments (< 300 bp), for analysing the middle part of the cytochrome *b* gene: CB_Frag2_F 5′-CAGTCGCACATATCTGTCG, CB_Frag2_R 5′-TGATGAATGGGAGGATAAAGTGG, CB_Frag3_F 5′-AGAATGAATCTGAGGCGGA, CB_Frag3_R 5′-ATTGATCGTAGGATTGCGT, CB_Frag4_F 5′-CTAGCACTAACCTTATTCACCC, CB_Frag4_R 5′-TGGCCTCCAATTCATGTTAGG. Difficult PCR (polymerase chain reaction) were carried out at least twice and along with two

**Table 1** The microsatellite loci used in this study

| Locus | Repeat* | Primer sequences (5′–3′)† | $T_a$ (°C) | Range (bp) | A |
|---|---|---|---|---|---|
| Lobs_Di7.1 | $(TG)_3/(TG)_4/(TG)_2/(TG)_{19}$ | F: ATCAGGGAGAGGTGAGAAGGGC<br>R: GTTTCTTCCTTGCTTAGTCTTTTGCTACCTTA | 63 | 118–152 | 17 |
| Lobs_Di9 | $(TG)_{16}$ | F: CAGTGAAGCAATGAAGAG<br>R: GTTTCTTAGATGACTGACTTGAAGGAG | 53 | 86–112 | 13 |
| Lobs_Di19 | $(CA)_{11}$ | F: CCCAAAATAAAACTGATGAGCAG<br>R: GTTTCTTGGTAGAGTCACAGTGTGTGC | 58 | 86–128 | 18 |
| Lobs_Di21 | $(TG)_{15}$ | F: CCTGGTGGCTGTCATTTGTGGAATA<br>R: GTTTCTTCTGTACTCCCTTGGGGGCAAAC | 63 | 98–128 | 16 |
| Lobs_Di24 | $(GT)_9(GA)_{10}$ | F: CCTCACTCAGGGGGAAATGGATTTA<br>R: GTTTCTTGCTACTAAAATTGGACTCCCTGGAG | 63 | 102–130 | 15 |
| Lobs_Di39 | $(CA)_{21}$ | F: ATTAAAACACTGATAACCCCGGACA<br>R: GTTTCTTAAAAGCTATTTTGTGCTGTCACCTA | 63 | 90–110 | 9 |
| Lobs_Di45 | $(CA)_{13}$ | F: ATTTTGCAAACACAAGTG<br>R: GTTTCTTCTACTATTTGAATGAAAAGAGAG | 53 | 94–112 | 10 |
| Lobs_Di47 | $(TG)_{11}/(CG)_3$ | F: TAGGGAGCCTATGTAAGACTTA<br>R: GTTTCTTCAGGTTTACAGAATAGGACTTATTT | 58 | 98–106 | 5 |
| Lobs_TT6 | $(AAAT)_5$ | F: AAACAAAGACCCACCACA<br>R: GTTTCTTCTCTTAATCTTAACATATTCCATAT | 53 | 80–92 | 4 |

*The 'repeat' column shows the structure of the repeat region in the cloned allele, with a forward slash signifying intervening unique sequence; †For each locus, the forward (F) primer was fluorescently labelled and the reverse (R) primers include a GTTTCTT tail (underlined) at its 5′-end to force A + alleles and, hence, improve binning of alleles. $T_a$, annealing temperature; Range, range of alleles sizes observed; A, observed number of alleles (across all samples).

negative controls to check for contamination both during the extraction and amplification procedures. Forty-one sequences new to this study were aligned with already published dusky dolphin haplotypes (Cassens *et al.* 2003), and a cytochrome *b* alignment of 795 bp, encompassing a total of 215 individuals, was used in all analyses. In addition, a portion of the mitochondrial control region (591 bp) was PCR-amplified and directly sequenced with the primers L15926 (modified from (Kocher *et al.* 1989)) and H00034 (from (Rosel *et al.* 1994)) for 153 individuals in order to compare its genetic variability with that of the cytochrome *b*. The presence of double peaks in some sequences indicated that nuclear copies of mitochondrial genes are frequently amplified (Cassens & Milinkovitch, in preparation). Therefore, to avoid amplification of shorter nuclear copies, we sequenced, in a few individuals, the cytochrome *b* gene and the control region on a much longer mitochondrial fragment (about 3.9kb) first amplified with the Expand™ Long Template PCR system (Roche) using an annealing temperature at 62 °C and the following primers: Long_tRNAGlu-For 5′-GTCTCACATGGACTYYAACCA-TGACCAATGA, Long_12S-Rev 5′-GGGTTTATCGRTTAY-AGAACAGGCTCCTCT. All new mitochondrial haplotypes (cytochrome *b* and control region) have been deposited in GenBank under accession numbers [AY821635–AY821653 (cytb) and AY821573-AY821634 (control region)].

Nuclear DNA variation was assayed using nine species-specific microsatellite loci that we isolated from a genomic library of *Lagenorhynchus obscurus* using the following procedure: genomic DNA was partly digested with Sau3AI and a fraction of the resulting DNA fragments ranging from 400 bp to 800 bp was isolated after electrophoresis in an agarose gel. The fragments corresponding to the selected fraction were purified, ligated to a zero background vector and transformed into competent cells. Clones were hybridized with oligonucleotide probes specific to dinucleotide, tri nucleotide and tetranucleotide repeats. Recombinant DNA molecules were isolated and sequences of inserted genomic DNA fragments were obtained by cycle sequencing and electrophoresis on an ABI 377 (Applied Biosystems). The full sequence of each positive clone was fed into the program OLIGOFAKTORY (unpublished, Laboratory of Evolutionary Genetics, Université Libre de Bruxelles) that designs optimal (i.e. minimizing dimer and hairpin interactions and maximizing specificity) primers flanking the repeated sequence. We screened 12 individuals for variation at 12 microsatellite loci and selected nine loci (Table 1) on the basis of variability and clarity of amplification patterns. Unique sequences of the cloned alleles are available from GenBank (AY821564-AY821572). The 5′-end of the forward primer from each selected locus set was fluorescently labelled. Reverse primers were designed with a GTTTCTT tail to reduce variability in adenylation of amplification products and thereby improve genotyping consistency and allele binning (Brownstein *et al.* 1996). Each PCR reaction contained: 1 × PCR buffer (50 mM Tris/HCl, pH 8.3),

2–4 mM $MgCl_2$, 1 mM dNTP, 0.6 μM of each primer, 0.75 U of FastStart *Taq* DNA polymerase (Roche Molecular Biochemicals), and 1 μL (~10–50 ng) of genomic DNA in a final volume of 25 μL. For difficult DNA extractions (i.e. from bone, cartilage, and decayed tissue samples), 0.8–2.0 mg/mL BSA was added to the PCR reaction. All loci were amplified with an initial denaturation step at 94 °C for 5min; followed by 25–39 cycles (depending on the locus and/or template quality) at 94 °C for 30 s, 53–63 °C for 30 s, 72 °C for 1 min; and a final extension at 72 °C for 45 min. PCR products from a total of 216 individuals were separated electrophoretically using an Applied Biosystems 3100 automated sequencer. Allelic sizes were scored against the size standard GS500 LIZ (Applied Biosystems) and analysed using the GENESCAN 3.7 and GENOTYPER 3.7 software (Applied Biosystems).

For samples with unknown gender, PCR-based sex determination was performed using the four primers described by (Rosel 2003). PCR products were separated by electrophoresis on 2% agarose (Biozym) gels and gender was determined from the resulting banding patterns.

### *Genetic diversity*

For each mtDNA data set (cytochrome *b* and control region), we used the program ARLEQUIN, v.2.000 (Schneider *et al.* 2000) to identify the number of haplotypes, and calculate haplotypic diversity ($H$) and nucleotide diversity ($\pi$) within populations. For microsatellite data, we tested for significant heterozygote deficiency per locus and per population (with the exception of the New Zealand 'population' that has been excluded from all equilibrium tests because of its small size) using a Hardy–Weinberg exact test based on Markov chain iterations (Guo & Thompson 1992). The assumption of independence within each possible pair of loci was assessed using a likelihood-ratio statistic, whose distribution is obtained by a permutation procedure (Slatkin & Excoffier 1996). All these tests were done with ARLEQUIN, version 2.000 (Schneider *et al.* 2000) and critical significance levels for multiple testing were corrected following the sequential Bonferroni procedure (Rice 1989). The following statistics of nuclear genetic variation within populations were computed as averages over the nine microsatellite loci with the software GEN-SURVEY (Vekemans & Lefèbvre 1997): number of alleles per locus ($K$); observed heterozygosity ($H_O$); and gene diversity ($H_E$), computed according to (Nei 1987). We also examined allelic richness ($A$) that takes into account unequal sample sizes using the rarefaction method (El Mousadik & Petit 1996): Allelic richness was calculated for the smallest number of individuals typed for a locus in a sample as implemented in FSTAT, version 2.9.3 (Goudet 1995). To assess the impact of recent demographic changes on genetic diversity, we used the heterozygosity excess test of (Cornuet & Luikart 1996). Indeed, populations that have experienced a recent reduction of their effective population size see their allelic diversity reduce faster than their heterozygosity. Heterozygosity excess was estimated based on 10 000 replications and tested for significance (one-tailed Wilcoxon test) using the program BOTTLENECK, v.1.2.02 (Piry *et al.* 1999). Using the same software, we examined the distribution of allele frequencies for a so-called 'mode-shift' that discriminates recently bottlenecked from stable populations (Luikart *et al.* 1998).

### *Population structure and dispersal behaviour*

To estimate phylogeographical structure, we inferred a median-joining graph (Bandelt *et al.* 1999) for both the control region and cytochrome *b* gene data sets using the program NETWORK, v.2.0 (available at http://www.fluxus-engineering.com/sharenet.htm). For each mtDNA data set, we estimated genetic differentiation among predefined populations in terms of pairwise $F_{ST}$-values based on genetic distances among haplotypes (using absolute number of differences). For microsatellite data, we first used an allele-size permutation procedure to test whether microsatellite allele sizes are informative with respect to genetic differentiation (Hardy *et al.* 2003) as implemented in SPAGEDI, version 1.1 (Hardy & Vekemans 2002). Nonrejection of the null hypothesis ($F_{ST} = R_{ST}$) led to the calculation of genetic differentiation between each pair of populations using $F_{ST}$ values (Weir & Cockerham 1984) as $F_{ST}$ is more appropriate than $R_{ST}$ when differentiation is caused mainly by drift. We also examined fine-scale genetic structure at the nine microsatellite loci, comparing dusky dolphins from Namibia ($N = 15$) and South Africa ($N = 46$) in Southeast Atlantic waters, and from the two adjacent harbours Pucusana ($N = 105$) and Cerro Azul ($N = 13$) in Peruvian waters (cf. Fig. 1b,c). All $F$-statistics estimations were performed using the ARLEQUIN software (Schneider *et al.* 2000).

To test whether our a priori definition of populations (based on the geographical location of sampled individuals) is consistent with genetic information, we also applied two Bayesian approaches to our microsatellite data set. The program BAPS, version 2.0 (Corander *et al.* 2003) jointly estimates the posterior probabilities for the following parameters: the number of clusters with different allele frequencies that ranges between one and the number of so-called 'sampling units' (e.g. different geographical sampling locations or individuals, see below), the partition of sampling units among the inferred clusters, and the relative allele frequencies. Two initial sampling unit levels have been specified: while the individual-level analysis (each of the 216 dusky dolphin individuals represented a different sampling unit) necessitated an MCMC (Markov Chain Monte Carlo) analysis (several runs of 50 000 iterations with a burn-in of 10 000, and sampling every five

generations), a second run with individuals pre-assigned to six subpopulations (Peru-Pucusana, Peru-Cerro Azul, Argentina, Namibia, South Africa, and New Zealand) could be performed by enumerative calculation. In addition, we used the program STRUCTURE, version 2.0 (Pritchard *et al.* 2000) for estimating the number of populations represented by the six sample locations to give us a separate insight into how genetic variation is organized. STRUCTURE uses a Bayesian MCMC approach to cluster individuals into groups while minimizing Hardy–Weinberg disequilibrium and gametic phase disequilibrium between loci within groups. The number of populations ($K$) most compatible with the observed data can be obtained by maximizing the estimated log-likelihood of the data for different values of $K$ (Pritchard *et al.* 2000). We performed a series of independent runs for $K$ from one to six populations, assuming correlated allele frequencies and an admixture model with an estimated proportion $\alpha$ of admixed individuals, with a burn-in of 500 000 iterations and a data collection period of $3 \times 10^6$–$1 \times 10^7$ iterations. Three runs for each value of K were performed to check for convergence.

To discriminate the relative importance of gene flow and genetic drift as explanations for observed levels of differentiation among populations, we used a recently developed MCMC method for the analysis of genetic data under a general 'isolation with migration' model (IM) (Nielsen & Wakeley 2001; Hey & Nielsen 2004). Under this model, a population gives rise to two populations of constant size which can then exchange alleles. Using control-region haplotypes found in Atlantic waters (given that fluctuations in population size are likely to have occurred in the Southeast Pacific, Peruvian individuals were excluded from the analysis), the posterior probabilities for six population parameters have been estimated simultaneously as implemented in the program IM: the sizes of the Argentine, southern African, and their ancestral population ($\theta_1 = 4N_1u$, $\theta_2 = 4N_2u$, $\theta_A = 4N_Au$); the two migration rates ($m_1 = m_1/u$ and $m_2 = m_2/u$); and the time of population splitting ($t = tu$); all of them scaled by the mutation rate u. To ensure a sufficient mixing of parallel MCMC chains and convergences of the estimates, we repeated runs three times (search parameters used: t30, m15, m25, q1100, b500 000, l$10^7$, n10, fg, g10.8, and g20.01).

To test for possible bias in dispersal between male and female dusky dolphins, we analysed our microsatellite data set using two different statistics. First, as we expect measures of genetic differentiation in the more philopatric sex to be higher than those in the more dispersing sex, sex-specific $F_{ST}$ values (Weir & Cockerham 1984) were compared. Second, we used individual assignment indices (first introduced by (Paetkau *et al.* 1995) and later modified by (Favre *et al.* 1997)) that calculate, for each individual, the probability of assigning its multilocus genotype to each population. Because members of the dispersing sex will include residents (with common genotypes) and immigrants (with rare genotypes), the variance of the assignment index (vAIc) for the sex dispersing most should be largest. Dusky dolphin populations off Peru, Argentina, and southern Africa were compared pairwise. A randomization approach (10 000 permutations) was used to test whether the statistics differed significantly between the two sexes, as implemented in FSTAT, version 2.9.3 (Goudet 1995). Given the polygynous mating system and other life-history characteristics of dusky dolphins, it is predicted that males are more prone to disperse and therefore only one-tailed tests have been carried out.

## Results

### *Genetic diversity*

*Mitochondrial DNA.* Cytochrome *b* gene sequences were compared among 215 dusky dolphins sampled from four populations (Peru, Argentina, southern Africa, and New Zealand). Of the total of 795 nucleotides scored, 53 sites are polymorphic and define 48 distinct haplotypes. A total of 62 haplotypes are identified among 153 dusky dolphin individuals that have additionally been sequenced at the 5′-end of the control region (591 nucleotides analysed, 64 variable sites). The single observed gap in the control region alignment was treated as missing data and recoded as a present/absent character at the end of the data matrix. Overall sequence variability is higher in the control region than in the cytochrome *b* fragment (with average haplotypic/nucleotide diversity of 0.97/1.63% and 0.89/0.56%, respectively; Table 2). Populations considerably differed in levels of genetic variation only at the cytochrome *b* gene: haplotypic diversity was lowest in Peruvian waters (0.68), whereas it ranged from 0.87 in Argentina to 0.93 in New Zealand. Nucleotide diversity varied from 0.15% in Peru to 0.95% in New Zealand (Table 2).

*Microsatellites.* All nine nuclear loci are polymorphic within each of the four analysed populations. After adjusting the significance level for multiple comparisons, all of the 27 population/locus combinations are in Hardy–Weinberg equilibrium and there is no evidence of linkage disequilibrium between loci. All populations show comparable levels of nuclear genetic diversity (Table 2): the allelic richness, averaged across the nine loci and calculated for six genotyped individuals from the whole sample, is 5.38 and ranges from 5.11 in New Zealand to 5.27 in Argentina, whereas the expected mean heterozygosity across all populations is 0.716 and ranges from 0.694 in New Zealand to 0.735 in the southern African population. Analyses of heterozygosity excess and of allele frequency distribution mode-shift did not detect any significant recent bottleneck in any of the four populations (Table 2).

**Table 2** Summary of genetic variability statistics for (a) the mitochondrial cytochrome *b* gene (b) the mitochondrial control region, and (c) nuclear microsatellite data

| Populations | All | Peru | Argentina | Southern Africa | New Zealand |
|---|---|---|---|---|---|
| (a) mtDNA-cytochrome *b* | | | | | |
| *N** | 215 | 119 | 29 | 61 | 6 |
| No. of haplotypes | 48 | 13 | 10 | 22 | 5 |
| *H*† | 0.89 ± 0.02 | 0.68 ± 0.04 | 0.87 ± 0.03 | 0.90 ± 0.02 | 0.93 ± 0.12 |
| π (in percentage)‡ | 0.56 ± 0.31 | 0.15 ± 0.10 | 0.40 ± 0.23 | 0.58 ± 0.32 | 0.95 ± 0.56 |
| (b) mtDNA-control region | | | | | |
| *N* | 153 | 118 | 14 | 21 | |
| No. of haplotypes | 62 | 38 | 12 | 13 | |
| *H* | 0.97 ± 0.01 | 0.95 ± 0.01 | 0.97 ± 0.04 | 0.96 ± 0.02 | |
| π (in percentage) | 1.63 ± 0.83 | 1.23 ± 0.64 | 1.23 ± 0.69 | 1.43 ± 0.77 | |
| (c) microsatellite data | | | | | |
| *N* | 216 | 120 | 29 | 61 | 6 |
| *K* | 8.25 | 10.8 | 8.1 | 9.0 | 5.1 |
| *A* | 5.38 | 5.16 | 5.27 | 5.14 | 5.11 |
| $H_O$ | 0.7262 | 0.7009 | 0.7122 | 0.7325 | 0.7593 |
| $H_E$ | 0.7162 | 0.7012 | 0.7340 | 0.7350 | 0.6944 |
| *P* (H excess)/mode shift¶ | | 0.87/no | 0.33/no | 0.71/no | 0.13/no |

**N*, number of dolphins analysed; †*H*, haplotypic diversity; ‡π, nucleotide diversity per site; For microsatellites, mean values (across nine loci) are given for the number of alleles per locus (*K*), the allelic richness (*A*) expected for six genotyped individuals (i.e. the sample size of the New Zealand sample), and the observed ($H_O$) and expected ($H_E$) heterozygosity per locus; ¶Results of two bottleneck tests are shown at the bottom of the table: *P*-values for heterozygosity excess (one-tailed Wilcoxon) test under the TPM model with 10% multistep changes, and presence or absence (no) of a mode-shift in allele frequency distributions.

## *Population structure and dispersal behaviour*

*Phylogeographical patterns.* The median-joining reticulated graphs for the cytochrome *b* gene and control region indicate that mitochondrial dusky dolphin haplotypes can clearly be separated into two major haplogroups (Fig. 2): the first comprising all Peruvian individuals, and the second corresponding to all other populations. Note however, that one Argentine haplotype (p2) falls into the Peruvian control-region haplogroup, suggesting that this individual is a recent migrant (Fig. 2b). No clear phylogeographical structuring is observed among haplotypes from Argentina, southern Africa and New Zealand. The two reticulated graphs differ in one important aspect: while internal node haplotypes of the cytochrome *b* gene are often present in high frequencies (and are sometimes found in more than one population, e.g. a1.1), many of the control-region lineages, especially in Atlantic waters, are connected to central missing intermediates indicating that ancient haplotypes have been replaced by more recent haplotypes (and no control-region haplotype is shared between Argentine and southern African waters). Surprisingly, haplotypes from New Zealand are scattered among Atlantic haplotypes, most often at the tip of distantly related lineages.

*F-statistics.* Based on microsatellite markers, no significant population structuring was found on a local geographical scale: neither between dusky dolphins from different Peruvian harbours nor between Namibian and South African individuals ($F_{ST}$ of 0.004 and −0.001, respectively). On the other hand, all three markers analysed (cytochrome *b*, control region, and microsatellites) indicate that the Peruvian, Argentine, and southern African populations are significantly different from each other (Table 3). For the comparisons Peru vs. Argentina and Peru vs. southern Africa, $F_{ST}$ values are surprisingly low at the nuclear

**Table 3** Genetic differentiation in terms of pairwise *F*-statistics. Above diagonal, genetic distance-based $F_{ST}$ values are given for mtDNA sequence data: first line, control region; second line, cytochrome *b* gene. Below diagonal, $F_{ST}$ values are given for the nine nuclear microsatellite loci. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, $P > 0.05$

| | Peru | Argentina | Southern Africa | New Zealand |
|---|---|---|---|---|
| Peru | | 0.435*** | 0.462*** | / |
| | | 0.726*** | 0.602*** | 0.735*** |
| Argentina | 0.035*** | | 0.082** | / |
| | | | 0.043* | 0.288** |
| Southern Africa | 0.039*** | 0.019*** | | / |
| | | | | 0.148* |
| New Zealand | 0.013ns | −0.002ns | 0.014ns | |

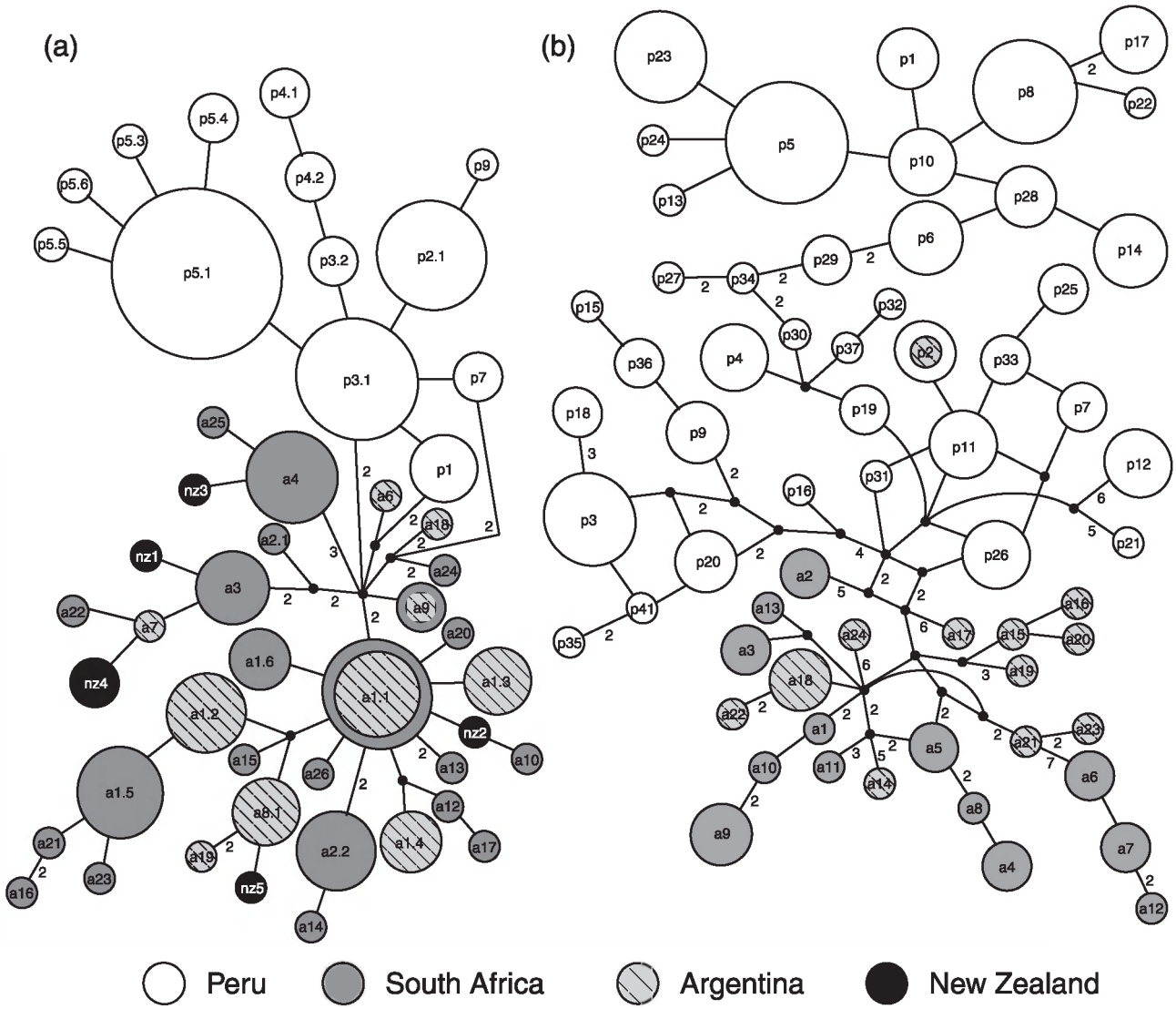


**Fig. 2** Genealogical relationships among cytochrome *b* gene (a) and control region (b) haplotypes as inferred by the median-joining algorithm. Circle size is proportional to the number of individuals exhibiting the corresponding haplotype. Small unlabelled black circles represent missing node haplotypes. If not otherwise stated by numbers, a branch connecting two haplotypes corresponds to a single mutational step.

microsatellite loci. Overall genetic differentiation is lowest across the Atlantic Ocean (i.e. between the Argentine and southern African populations), especially for mitochondrial data. Interestingly, however, the significance level for the population pair Argentina vs. southern Africa increases with the increasing mutation rate of the marker (lowest for the cytochrome *b* gene, intermediate for the control region, and highest for microsatellites). Using microsatellites, the population off New Zealand could not be genetically differentiated, whereas significant $F_{ST}$ values (from 0.148 to 0.735 for New Zealand vs. southern Africa and New Zealand vs. Peru, respectively) are observed when cytochrome *b* sequences are compared.

*Bayesian clustering approaches.* No clear nuclear structure could be detected among sampling locations analysing our microsatellite data set with the two Bayesian clustering approaches. Of all possible combinations of sampling localities, the Bayesian analysis of population structure (BAPS) (Corander *et al.* 2003) finds the highest posterior probability ($P = 0.9995$) for a structure where southern Africa (South Africa + Namibia) is a separate population while the other four geographical locations (Peru-Pucusana, Peru-CerroAzul, Argentina, New Zealand) cluster together. The maximum probability of other partitions is 0.01. The individual-level analyses (each individual represents a different sampling unit), however, revealed a lack of

**Table 4** Results of three independent Bayesian cluster analysis runs (I–III) with various values of the parameter $K$ (number of populations specified a priori)

| $K$-value (MCMC iterations)* | Estimated ln likelihood: ln Pr($X/K$) | | | Variance in ln likelihood | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| 1 ($3 \times 10^6$) | −6930.2 | −6931.4 | −6931.0 | 47.8 | 49.2 | 48.9 |
| 2 ($3 \times 10^6$) | −6817.6 | −6821.6 | −6819.2 | 247.9 | 254.9 | 250.8 |
| 3 ($3.5 \times 10^6$) | −6917.6 | −6867.5 | −6897.4 | 555.1 | 478.2 | 517.1 |
| 4 ($10^7$) | −7233.5 | −7753.5 | −7567.9 | 1334.9 | 2332.8 | 1988.8 |
| 5 ($8 \times 10^6$) | −6969.1 | −7028.9 | −7011.8 | 838.6 | 964.3 | 910.1 |
| 6 ($8 \times 10^6$) | −7276.3 | −7171.5 | −7116.2 | 1460.1 | 1239.1 | 1110.6 |

*The number of MCMC iterations is indicated in brackets (burnin always = $0.5 \times 10^6$).

**Table 5** Proportion of individuals from each sample location assigned to each of the two clusters inferred from the 'STRUCTURE' analysis (see text for details)

| Sampling location (sample size) | Inferred population clusters | |
|---|---|---|
| | 1 | 2 |
| Peru-Pucusana (105) | 0.157 | **0.843** |
| Peru-Cerro Azul (13) | 0.084 | **0.916** |
| Argentina (29) | 0.375 | **0.625** |
| South Africa (15) | **0.723*** | 0.277 |
| Namibia (15) | **0.777** | 0.223 |
| New Zealand (6) | 0.281 | **0.719** |

*Proportions greater than 0.5 are shown in bold.

phylogeographical structuring with many inferred clusters (up to 11), most of them containing either a single specimen or individuals from several different sampling locations. Likewise, the STRUCTURE program suggested that the individuals from the six sampling locations represent most likely only two different groups [highest ln Pr($X/K$) estimates for $K = 2$ in Table 4], although the large associated variances make this result not significant. Differences among sampling areas exist with respect to assignment of individuals to these two groups, again differentiating Namibia and South Africa from all other populations (Table 5). However, a high degree of admixture is also indicated in this analysis as (i) only for $K = 1$ and 2, estimates of ln Pr($X/K$) reasonably converged across runs, while all Bayesian clustering analyses for $K = 3$, 4, 5, and 6 produced estimates that were less consistent across runs and had very high variances (Table 4), and (ii) alpha values did not stabilize to relatively constant values during runs and among multiple runs for the same value of $K$, suggesting no clear population structure (Pritchard *et al.* 2000).

*Population differentiation in Atlantic waters: an analysis under the IM model.* The positions of the peaks of the marginal posterior densities for the population size parameters imply that (i) the ancestral dusky dolphin population that gave rise to the Argentine and southern African populations was very small, and (ii) the Argentine population has an effective population size about four times that of the southern African population (Fig. 3a). Furthermore, the analysis suggests that the two Atlantic populations diverge without any migration between them (both migration rate parameters are estimated to be zero; Fig. 3b). Finally, an estimation of the scaled time parameter (Fig. 3c) indicates a divergence time $t$ of 4.7. Because the corresponding absolute time $t = \mathrm{tu}$ and $\theta = 4Nu$, we can obtain an estimate of time in units of $2N$ generations by dividing the estimate of $t$ by one half of the estimate of $\theta$ as described in (Hey *et al.* 2004). Unfortunately, little information is available on the abundance of *Lagenorhynchus obscurus* throughout its range. However, using data from (Schiavini *et al.* 1999), we can estimate the effective population size off the Argentine coast to be smaller than 20 000. With $\theta_1 = 185$, this leads to an estimate of time of $4.7/(185/2) = 0.051$, in units of two N1 generations, where N1 is the population size of the Argentine population. With an average generation time of 10 years, our analysis yields then an estimate of 20 000 years divergence between the Argentine and southern African populations.

*Gender-biased dispersal.* For a total of 120 dusky dolphins from Peru ($N_{male} = 55$; $N_{female} = 65$), Argentina ($N_m = 8$; $N_f = 15$), and southern Africa ($N_m = 27$; $N_f = 27$), we determined gender either morphologically or by means of molecular data (cf. Materials and methods section). As expected with male-biased gene flow, the variance of the corrected assignment indices (*vAIc*) was higher for males in all population comparisons (Table 6). These differences were however, significant only when the Peruvian and Argentine populations were used. On the contrary, no sex-biased dispersal could be detected using $F$-statistics.

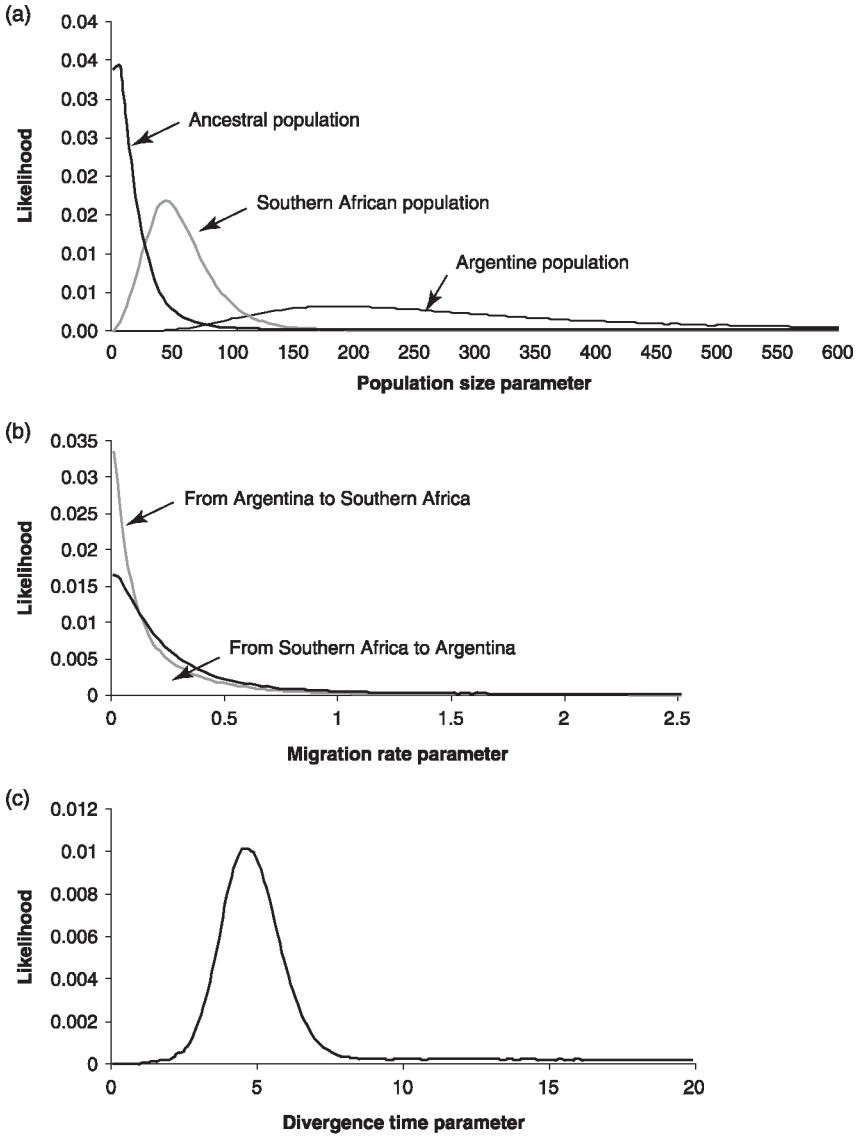


**Fig. 3** Population sizes, migration rates, and divergence time in Atlantic dusky dolphins. The marginal densities for parameters were obtained by fitting the 'isolation with migration' model (IM) to the mitochondrial control region data set. The probability functions are plotted for (a) the population size parameters ($\theta_1$, $\theta_2$, $\theta_A$) (b) migration parameters ($m_1$, $m_2$), and (c) the divergence time parameter ($t$).

## Discussion

The use of multiple marker systems and different analytical approaches provides valuable insights into the processes that have shaped the geographical distribution of genetic diversity exhibited by contemporary dusky dolphin populations. We will, respectively, discuss the genetic diversity levels observed in the Peruvian population, the possible dispersion patterns along the South American coast and across the Atlantic, the fine-scale geographical analyses, and the relationships between dusky dolphins from New Zealand and other populations. While a comprehensive assessment of stock structure is critical for the effective conservation of populations in this delphinid species, we suggest that our study offers also a good empirical example of natural, nonequilibrium, populations where the discrimination of the relative importance of distinct evolutionary forces, variable both in time and space, is challenging.

### *Ancient vs. recent reduction of genetic diversity in Peruvian dusky dolphins*

In extensively exploited cetacean populations, there is great concern that human-induced high mortality levels may have significantly reduced genetic variation (Baker *et al.* 1993; Caswell *et al.* 1999; Rooney *et al.* 1999; Pichler & Baker 2000). Peruvian dusky dolphins might have experienced such losses of genetic variability because small cetaceans off Peru have been severely affected by direct takes for three decades (Van Waerebeek & Reyes 1990, 1994a; Van Waerebeek *et al.* 1997). Furthermore, Peruvian

**Table 6** Gender-biased dispersal estimated in terms of $F_{ST}$ values and variance of corrected assignment indices (*vAIc*)

| | All | Peru-Argentina | Peru-Southern Africa | Argentina-Southern Africa |
|---|---|---|---|---|
| $F_{ST}$ (male) | 0.043 | 0.024 | 0.053 | 0.009 |
| $F_{ST}$ (female) | 0.035 | 0.039 | 0.037 | 0.020 |
| *P*-value | 0.716 | 0.176 | 0.900 | 0.213 |
| *vAIc* (male) | **15.519*** | **18.349** | 15.412 | 10.654 |
| *vAIc* (female) | **10.007** | **10.184** | 10.764 | 7.994 |
| *P*-value | **0.038** | **0.025** | 0.077 | 0.241 |

*Comparisons with significant differences between sexes are in bold.

dusky dolphins live in an extremely unstable environment where El Niño events recurrently cause increased rates of mortality among marine organisms, with the most severe oscillations of the last century having occurred in 1982–1983 and 1997–1998 (Berta & Sumich 1999). Although the lower cytochrome *b* haplotypic and nucleotide diversities in Peruvian than in other dusky dolphin populations [Table 2 and (Cassens *et al.* 2003)] is compatible with a general demographic decline of the Peruvian population, our analyses rather hint at an ancient bottleneck that predates recent El Niño oscillations and human exploitation. For instance, when investigating microsatellite allelic distribution, no signature of a recent reduction in population size is detected using heterozygosity excess or mode shift analyses (Table 2), although this negative result might also be explained by the use of less than 20 loci [a typical suggested target number required to achieve high power for detecting departures from mutation-drift equilibrium; (Cornuet & Luikart 1996)]. Furthermore, genetic variability levels within the Peruvian population is not lower than those of the other populations neither for the nine nuclear microsatellite loci nor, more importantly, for the mitochondrial control region (Table 2). Not only are microsatellite loci more likely than mitochondrial sequences to retain genetic diversity [resulting from the fourfold higher effective population size for autosomal markers in diploid organisms; (Birky *et al.* 1989)], but also their high mutation rate may preclude the possibility to detect an ancient low-diversity signature. The contrasting patterns of genetic variation in the two mitochondrial fragments (Fig. 2) — that are physically linked but show different mutation rates — rather suggest an ancient reduction of genetic variability in Peruvian waters: the relatively slow-evolving mitochondrial cytochrome *b* gene retains low diversity levels within the Peruvian population whereas that signature is already obscured by mutations in more rapidly evolving markers such as the 5′ end of the mitochondrial control region.

### *Population structure and dispersal pattern along the South American coast*

Our mitochondrial DNA sequence analyses reveal high genetic differentiation of the Peruvian dusky dolphin population. High pairwise $F_{ST}$ values including Peruvian dusky dolphins (Table 3) and the unambiguous identification of a Peruvian lineage in the median-joining graphs (Fig. 2) both suggest a very low rate of recent female dispersal between Peruvian and other populations. Given that the southeastern Pacific stock is threatened by high human-induced and natural mortality rates (cf. Introduction & Discussion sections), this finding has relevance for conservation as it indicates a poor ability of Peruvian dusky dolphins to recover via recruitment of nonindigenous females.

On the contrary, genetic differentiation among populations (including the Peruvian population) is much less pronounced for the nine autosomal microsatellite loci (Table 3). Contrasting patterns between nuclear and mitochondrial DNA in terms of *F*-statistics have often been interpreted as evidence for male-biased dispersal that homogenizes allele frequencies among populations at biparentally, but not at maternally, inherited genetic markers (Baker *et al.* 1998; Lyrholm *et al.* 1999; Escorza-Trevino & Dizon 2000). However, such observed differences in population structure at nuclear vs. mitochondrial loci can also be explained by the high rates of mutation at microsatellite loci: for example, $F_{ST}$ values less than one and estimates of gene flow greater than zero can be obtained even if the populations share no allele (Hedrick 1999; Neigel 1997). Moreover, fundamental problems remain when using mere magnitude of genetic differentiation to infer migration rates among populations, especially in non-equilibrium populations (Whitlock & McCauley 1999). For example, measures of genetic differentiation such as *F*-statistics are sensitive to fluctuating effective population sizes and can considerably increase when one or both populations go through a bottleneck (Zhivotovski 2001). As already mentioned above, this effect is expected to be more pronounced in mitochondrial markers as effective population size should be one-fourth that of a nuclear locus in diploid species. Therefore, given that the Peruvian population has probably experienced one or multiple bottlenecks (hence, increased genetic drift) in the past, it is not entirely surprising that the difference between nuclear and mitochondrial $F_{ST}$ values is much lower for the Argentina vs. the southern Africa population comparisons than for the Peruvian vs. each of the Atlantic populations (Table 3).

Independent of the previously-described issues, our analyses are nonetheless consistent with male-biased gene flow along the South American coast. First, the high level of significance for the difference in variance of assignment

indices (*vAIc*) between males and females (when comparing the two South-American populations; Table 6) is mostly resulting from a high male-*vAIc* (rather than from a low female-*vAIc*), suggesting a particularly large male-biased gene flow between Peruvian and Argentine waters. It should be noted that tests for detecting sex-biased dispersal have limited power unless the bias in dispersal is extreme, i.e. larger than four (Goudet *et al.* 2002). Moreover, the different performances of the two tests applied to our data (*vAIc* and *F*-statistics, the latter detecting no significant sex-specific dispersal) suggest that dispersal occurs at rates lower than 10%, because *vAIc* has been shown to perform better only below these migration rates (Goudet *et al.* 2002). Second, although Bayesian clustering methods (as implemented in BAPS and STRUCTURE) showed, especially in individual-based analyses, no clear overall population structure, both approaches were surprisingly consistent in suggesting that Argentine individuals are more closely related to Peruvian than to southern African dusky dolphins (see Results Tables 4 and 5). Finally, if sampling is sufficiently extensive, we expect to occasionally sample males that have dispersed. In this respect, it would be interesting to sex the single specimen in the Argentine population that showed a mitochondrial control region haplotype characteristic of the Peruvian haplogroup (p2 in Fig. 2). Unfortunately, we could not determine the gender, nor the cytochrome *b* haplotype, of that individual as a result of the very low quantity and bad quality of the DNA extracted from that sample. Male migration between Peruvian and Argentine waters might be surprising given that distances of about 3500 km need to be covered. However, long-range movements of more than 750 km along the Argentine coast have been recorded for two tagged dusky dolphins (Wuersig & Bastida 1986), and seasonal migrations have been described for several populations (Van Waerebeek 2002). Our results suggest that gene flow should certainly be taken into account when the ecological, morphological, and genetic divergence of Peruvian vs. Argentine dusky dolphins is discussed.

### *Population structure and dispersal patterns across the Atlantic*

Dusky dolphins are usually found in coastal waters over the continental shelf, but have also been recorded around many oceanic island groups. In the South Atlantic, for example, the occurrence of dusky dolphins in the vicinity of Gough Island might be explained by either a disjunct oceanic population or by recurrent migration between Argentine and southern African waters. At first sight, genetic evidence is consistent with the hypothesis that dusky dolphins regularly cross the pelagic waters of the southern Atlantic as (i) genetic differentiation in terms of *F*-statistics is low for all markers analysed (Table 3), and (ii) phylogeographical structuring is not clear among Atlantic cytochrome *b* nor control-region haplotypes (Fig. 2). However, when populations or species have recently separated, they often share ancient genetic variation. Therefore, it can be difficult to determine whether shared alleles are the result of gene flow or because of the persistence of ancient variation that originated prior to the divergence of the two populations. Our analyses provide stronger support for the latter hypothesis: the dusky dolphins off Argentina and southern Africa recently separated and, since then, diverge without considerable gene flow. The genealogical relationships among Atlantic haplotypes for the two mitochondrial gene fragments (Fig. 2) suggest that ancient variation is still present in both populations for the cytochrome *b* gene (e.g. central haplotype a1.1 in Fig. 2a), but has already been replaced by more recent control-region haplotypes. Without gene flow, the divergence between two populations is linearly increasing with time and variability of the marker (assuming neutrality, constant population sizes, etc.). Similarly, under the infinite-allele model (Kimura & Crow 1964), markers with higher mutation rates should provide higher resolution for differentiating the diverging populations, an expectation which is met by the Atlantic populations as the significance levels of the *F*-statistics increase with the increasing mutation rate of the three marker systems used in our study (Table 3).

Moreover, the use of the newly developed IM program (Nielsen & Wakeley 2001; Hey & Nielsen 2004; Hey *et al.* 2004) for the analysis of our mitochondrial control region data set allows further insight into the divergence of Argentine and southern African dusky dolphins. First, note that much larger population sizes have been estimated for both extant Atlantic populations compared with the ancestral one. As large and expanding populations are more likely to retain ancestral variation, this could partly explain why Atlantic dusky dolphins still show little differentiation. Second, both migration rates were estimated to be zero, not supporting the hypothesis of ongoing gene flow across Atlantic waters. And third, the conversion of the scaled time parameter led to an estimate that the Argentine and the southern African populations separated about 20 000 years ago (i.e. only about 2000 generations). Given the use of a single gene fragment and uncertain abundance data, these estimations should be interpreted with great caution. If our hypothesis is however correct, the Argentine and southern African dusky dolphin populations have evolved as independent evolutionary lineages since they separated.

### *Population substructuring and the phylogenetic position of New Zealand dusky dolphins*

Many studies that investigated genetic differentiation in cetaceans have indicated a complex pattern of population

structure, even at a low geographical scale (Hoelzel *et al.* 1998a,b; Rosel *et al.* 1994; Wang *et al.* 1999). Especially in areas of high impact from fisheries and other human activities, the putative presence of multiple genetic stocks that occur in parapatry or sympatry complicates the identification of management units. In the present study, fixation indices and Bayesian clustering approaches (BAPS and STRUCTURE) applied to our microsatellite data did not detect any genetic subdivision within Peruvian or southern African waters, a result which is consistent with a population genetic study carried out on New Zealand's dusky dolphin (Harlin *et al.* 2003).

Finally, the low genetic differentiation of New Zealand's dusky dolphins from the Atlantic populations is quite surprising. All five cytochrome *b* gene haplotypes found in New Zealand waters are not shared by other populations and are positioned at the tips of distantly related Atlantic lineages within the cytochrome *b* median-joining graph (Fig. 2a), consistent with the hypothesis that the New Zealand dusky dolphin population originated from multiple migration events from Atlantic waters. Note that there is no reason the low number of New Zealand samples should be responsible for that result.

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## References

Baker CS, Medrano-Gonzalez L, Calambokidis J *et al.* (1998) Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. *Molecular Ecology*, **7**, 695–707.

Baker CS, Perry A, Bannister JL *et al.* (1993) Abundant mitochondrial DNA variation and worldwide population structure in humpback whales. *Proceedings of the National Academy of Science, USA*, **90**, 8239–8243.

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Berta A, Sumich JL (1999) *Marine Mammals Evolutionary*. Biology Academic Press, San Diego, CA.

Bérubé M, Aguilar A, Dendanto Det al. (1998) Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci. *Molecular Biology*, **7**, 585–599.

Birky CW, Fuerst P, Maruyama T (1989) Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. *Genetics*, **121**, 613–627.

Brown Gladden JG, Ferguson MM, Friesen MK, Clayton JW (1999) Population structure of North American beluga whales (*Delphinapterus leucas*) based on nuclear DNA microsatellite variation and contrasted with the population structure revealed by mitochondrial DNA Variation. *Molecular Ecology*, **8**, 347–363.

Brownstein MJ, Carpten D, Smith JR (1996) Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: primer modifications that facilitate genotyping. *Biotechniques*, **20**, 1004–1010.

Cassens I, Van Waerebeek K, Best PB *et al.* (2003) The phylogeography of dusky dolphins (*Lagenorhynchus obscurus*): a critical examination of network methods and rooting procedures *Molecular Ecology*, **12**, 1781–1792.

Cassens I, Vicario S, Waddell VG *et al.* (2000) Independent adaptation to riverine habitats allowed survival of ancient cetacean lineages. *Proceedings of the National Academy of Science, USA*, **97**, 11343–11347.

Caswell H, Fujiwara M, Brault S (1999) Declining survival probability threatens the North Atlantic right whale. *Proceedings of the National Academy of Science, USA*, **96**, 3308–3313.

Chakraborty R, Nei M (1977) Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. *Evolution*, **31**, 347–356.

Cooper JE, Laurie WA (1987) Investigation of deaths in marine iguanas (*Amblyrhynchus cristatus*) in Galapagos. *Journal of Computer Pathology*, **97**, 129–136.

Corander J, Waldmann P, Sillanpää MJ (2003) Bayesian analysis of genetic differentiation between populations. *Genetics*, **163**, 367–374.

Cornuet J-M, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Marocco. *Theoretical and Applied Genetics*, **92**, 832–839.

Escorza-Trevino S, Dizon AE (2000) Phylogeography, intraspecific structure and sex-biased dispersal of Dall's porpoise, *Phocoenoides dalli*, revealed by mitochondrial and microsatellite DNA analyses. *Molecular Ecology*, **9**, 1049–1060.

Favre L, Balloux F, Goudet J, Perrin N (1997) Female-biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns. *Proceedings of the Royal Society, London*, **264**, 127–132.

Gilbert GS, Harms KE, Hamill DN, Hubbell SP (2001) Effects of seedling size, El Niño drought, seedling density, and distance to nearest conspecific adult on 6-year survival of *Ocotea whitei* seedlings in Panama. *Oecologia*, **127**, 509–516.

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using biparentally inherited genetic markers. *Molecular Ecology*, **11**, 1103–1114.

Guo SW, Thompson EA (1992) Permorming the exact test for Hardy–Weinberg proportions for multiple alleles. *Biometrics*, **48**, 361–372.

Hardy O, Charbonnel N, Fréville H, Heuertz M (2003) Microsatellite allele sizes: a simple test to assess their significance on genetic differentiation. *Genetics*, **163**, 1467–1482.

Hardy O, Vekemans X (2002) SPAGEDI: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Harlin AD, Markowitz TM, Baker CS, Würsig B, Honeycutt RL (2003) Genetic structure, diversity, and historical demography of New Zealand's dusky dolphins (*Lagenorhynchus obscurus*) *Journal of Mammology*, **84**, 702–717.

Hedrick PW (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.

Hey J, Nielsen R (2004) Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics*, **167**, 747–760.

Hey J, Won Y-J, Sivasundar A, Nielsen R, Markert JA (2004) Using nuclear haplotypes with microsatellites to study gene flow between recently separated Cichlid species. *Molecular Ecology*, **13**, 909–919.

Hillis DM, Moritz C, Mable BK (1996) *Molecular Systematics*, p. 655. Sinauer Associates, Inc., Sunderland, MA.

Hoelzel AR (1998) Genetic structure of cetacean populations in sympatry, parapatry, and mixed assemblages: implications for conservation policy. *Journal of Heredity*, **89**, 451–458.

Hoelzel AR, Dahlheim M, Stern SJ (1998a) Low genetic variation among killer whales (*Orcinus orca*) in the eastern North Pacific and genetic differentiation between foraging specialists. *Journal of Heredity*, **89**, 121–128.

Hoelzel AR, Potter CW, Best PB (1998b) Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. *Proceedings of the Royal Society, London*, **265**, 1177–1183.

Jefferson TA, Van Waerebeek K (2002) The taxonomic status of the nominal dolphin species *Delphinus tropicalis* van Bree, 1971. *Marine Mammal Science*, **18**, 787–818.

Kiesecker JM, Blaustein AR, Belden LK (2001) Complex causes of amphibian population declines. *Nature*, **410**, 639–640.

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. *Genetics*, **49**, 725–738.

Kingston SE, Rosel PE (2004) Genetic differentiation among recently diverged Delphinid taxa determined using AFLP markers. *Journal of Heredity*, **95**, 1–10.

Kocher TD, Thomas WK, Meyer A *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Science, USA*, **86**, 6196–6200.

Luikart G, Allendorf FW, Sherwin B, Cornuet J-M (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*, **12**, 238–247.

Lyrholm T, Leimar O, Johanneson B, Gyllensten U (1999) Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. *Proceedings of the Royal Society, London*, **266**, 347–354.

Milinkovitch MC, LeDuc RG, Tiedemann R, Dizon AE (2002) Applications of molecular data in cetacean taxonomy and population genetics with special emphasis on defining species boundaries. In: *Marine Mammals: Biology and Conservation* (eds Evans PGH, Raga JA), pp. 325–359. Kluwer Academic/Plenum Publishers, New York.

Natoli A, Peddemors VM, Hoelzel AR (2004) Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. *Journal of Evolutionary Biology*, **17**, 363–375.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York, USA.

Neigel JE (1997) A comparison of alternative strategies for estimating gene flow from genetic markers. *Annual Review of Ecological and Systematics*, **28**, 105–128.

Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov Chain Monte Carlo approach. *Genetics*, **158**, 885–896.

O'Corry-Crowe GM, Suydam RS, Rosenberg A, Frost KJ, Dizon AE (1997) Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the Western Nearctic revealed by mitochondrial DNA. *Molecular Ecology*, **6**, 955–970.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears in Alaska. *Molecular Ecology*, **4**, 347–354.

Perrin N, Akin PA, Kashiwada JV (1991) Geographic variation in external morphology of the spinner dolphin *Stenella longirostris* in the eastern Pacific and implications for conservation. *Fishery Bulletin*, **89**, 411–428.

Pichler FB, Baker CS (2000) Loss of genetic diversity in the endemic Hector's dolphin due to fisheries-related mortality. *Proceedings of the Royal Society, London B*, **267**, 97–102.

Piry S, Luikart G, Cornuet J-M (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**, 502–503.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rooney AP, Honeycutt RL, Davis SK, Derr JN (1999) Evaluating a putative bottleneck in a population of bowhead whales from patterns of microsatellite diversity and genetic disequilibria. *Journal of Molecular Evolution*, **49**, 682–690.

Rosel PE (2003) PCR-based determination in Odontocete cetaceans. *Conservation Genetics*, **4**, 647–649.

Rosel PE, Dizon AE, Heyning JE (1994) Genetic analysis of sympatric morphotypes of common dolphins (genus *Delphinus*). *Marine Biology*, **119**, 159–167.

Schiavini ACM, Pedraza SN, Crespo EA, Gonzalez R, Dans SL (1999) Abundance of dusky dolphins (*Lagenorhynchus obscurus*) off North and Central Patagonia, Argentina, in spring and a comparison with incidental catch in fisheries. *Marine Mammal Science*, **15**, 828–840.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN, version 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Sheppard CR, Spalding M, Bradshaw C, Wilson S (2002) Erosion vs. recovery of coral reefs after 1998 El Niño: Chagos reefs, Indian Ocean. *Ambio*, **31**, 40–48.

Slatkin M, Excoffier L (1996) Testing for linkage disequilibrium in genotypic data using the EM algorithm. *Heredity*, **76**, 377–383.

Van Waerebeek K (1993a) External features of the dusky dolphin *Lagenorhynchus obscurus* (Gray, 1828) from Peruvian waters. *Estudios Oceanologicos*, **12**, 37–53.

Van Waerebeek K (1993b) Geographic variation and sexual dimorphism in the skull of the dusky dolphin, *Lagenorhynchus obscurus* (Gray, 1828). *Fishery Bulletin*, **91**, 754–774.

Van Waerebeek K (1994) A note on the status of the dusky dolphin (*Lagenorhynchus obscurus*) off Peru. *Report of the International Whaling Commission (Special Issue)*, **15**, 525–527.

Van Waerebeek K (2002) Pacific white-sided dolphin and dusky dolphin — *Lagenorhynchus obliquidens* and *L. obscurus*. In: *Encyclopedia of Marine Mammals* (eds Perrin WF, Würsig B, Thewissen JGM), pp. 859–861. Academic Press, San Diego.

Van Waerebeek K, Read AJ (1994) Reproduction of dusky dolphins, *Lagenorhynchus obscurus*, from coastal Peru. *Journal of Mammalogy*, **75**, 1054–1062.

Van Waerebeek K, Reyes JC (1990) Catch of small cetaceans at Pucusana Port, Central Peru, during 1987. *Biological Conservation*, **51**, 15–22.

Van Waerebeek K, Reyes JC (1994a) Interactions between small cetaceans and Peruvian fisheries in 1988–1989 and analysis of trends. *Report of the International Whaling Commission (Special Issue)*, **15**, 495–502.

Van Waerebeek K, Reyes JC (1994b) Post-ban small cetacean takes off Peru: a review. *Report of the International Whaling Commission (Special Issue)*, **15**, 503–520.

Van Waerebeek K, Van Bree PJH, Best PB (1995) On the identity of *Prodelphinus petersii* Luetken, 1889 and records of dusky dolphin *Lagenorhynchus obscurus* (Gray, 1828) from the southern Indian and Atlantic Oceans. *South African Journal of Marine Science*, **16**, 25–35.

Van Waerebeek K, Van Bressem M-F, Felix F *et al.* (1997) Mortality of dolphins and porpoises in coastal fisheries off Peru and southern Ecuador in 1994. *Biological Conservation*, **81**, 43–49.

Vekemans X, Lefèbvre C (1997) On the evolution of heavy-metal tolerant populations in *Armeria maritima*: evidence from allozyme variation and reproductive barriers. *Journal of Evolutionary Biology*, **10**, 175–191.

Wang JY, Chou L-S, White BN (1999) Mitochondrial DNA analysis of sympatric morphotypes of bottlenose dolphins (genus: *Tursiops*) in Chinese waters. *Molecular Ecology*, **8**, 1603–1612.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. *Heredity*, **82**, 117–125.

Woodley TH, Read AJ (1991) Potential rates of increase of a harbour porpoise (*Phocoena phocoena*) population subjected to incidental mortality in commercial fisheries. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 2429–2435.

Wuersig B, Bastida R (1986) Long-range movement and individual associations of two dusky dolphins (*Lagenorhynchus obscurus*) off Argentina. *Journal of Mammalogy*, **67**, 773–774.

Zhivotovski LA (2001) Estimating divergence time with the use of microsatellite genetic distances: impacts of population growth and gene flow. *Molecular Biology and Evolution*, **18**, 700–709.

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