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Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*)

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Bottlenose dolphins (*Tursiops truncatus*) are widely distributed and a high degree of morphometric and genetic differentiation has been found among both allopatric and parapatric populations. We analysed 145 samples along a contiguous distributional range from the Black Sea to the eastern North Atlantic for mitochondrial and nuclear genetic diversity, and found population structure with boundaries that coincided with transitions between habitat regions. These regions can be characterized by ocean floor topography, and oceanographic features such as surface salinity, productivity and temperature. At the extremes of this range there was evidence for the directional emigration of females. Bi-parentally inherited markers did not show this directional bias in migration, suggesting a different dispersal strategy for males and females at range margins. However, comparative assessment based on mitochondrial DNA and nuclear markers indicated that neither sex showed a strong bias for greater dispersal on average. These data imply a mechanism for the evolutionary structuring of populations based on local habitat dependence for both males and females.

Keywords: bottlenose dolphin; population genetics; Mediterranean Sea; Black Sea; sex-biased dispersal

1. INTRODUCTION

The Mediterranean and Black Seas represent a unique marine ecosystem. Geographically they consist of a sequence of contiguous basins, separated from the Atlantic Ocean by a narrow strait at Gibraltar. Both the Strait of Gibraltar and the straits that connect the Mediterranean and Black Sea basins (the Turkish Strait System) have been suggested to represent a barrier to gene flow for some species (e.g. hake, *Merluccius merluccius* (Roldàn *et al.* 1998); sardines, *Sardinella aurita* (Chikhi *et al.* 1997); cuttlefish, *Sepia officinalis* (Perez-Losada *et al.* 2002); fin whales, *Balaenoptera physalus* (Bérubé *et al.* 1998); striped dolphins, *Stenella coeruleoalba* (Garcia-Martinez *et al.* 1999)). For other species, a more likely boundary to gene flow was identified as an oceanic front some 350 km to the east of the Strait of Gibraltar: the Almeria-Oran oceanic front, where the Atlantic oceanic waters encounter warmer and denser Mediterranean waters (e.g. sea bass, *Dicentrarchus labrax* (Naciri *et al.* 1999); Mediterranean mussels, *Mytilus galloprovincialis* (Quesada *et al.* 1995)). However, any structuring across these putative boundaries would have to have been recent, as the shape and connectivity of these basins have changed considerably over the course of the Holocene, and as recently as 7900 years ago (e.g. Ryan *et al.* 1997).

The Mediterranean Sea and Black Sea offer a wide variety of different oceanographic environments, ranging from very shallow waters and sandy floors in the Adriatic Sea to very deep abyssal areas in the Ionian Sea, and oceanographic discontinuities can be identified throughout the whole range (see figure 1). However, there are broader distinctions among regions. The Mediterranean Sea is generally characterized by higher salinity and higher water temperature compared with the Atlantic Ocean. The Black Sea is characterized both by low salinity, due to high outflow of fresh water from rivers, and low water temperature, especially during the winter when the water usually freezes in the northeast (e.g. in the Azov Sea; see UNEP 1996). The Mediterranean basin is physically divided in the region of Italy, with predominantly deep water to the west, and shallower water with more complex benthic topography to the east (see figure 1).

Bottlenose dolphins are observed throughout the geographical range of our study from Scotland to the Black Sea. In both inland seas, they commonly inhabit coastal areas (Notarbartolo di Sciara *et al.* 1993), although occasional sightings offshore and long-distance movements of over 200 km have been reported (Morozova 1981). The Black Sea bottlenose dolphin is considered by some to be an endemic subspecies named *Tursiops truncatus ponticus*, based especially on its smaller body size compared with bottlenose dolphins from other areas (Tomilin 1957; Hershkovitz 1966). Common dolphin and harbour porpoise populations in the Black Sea have also been proposed to be different subspecies (*Delphinus delphis ponticus* and *Phocoena phocoena relicta*; (Tomilin 1957; Hershkovitz 1966)).

The bottlenose dolphin shows strong population genetic structure among populations across its worldwide range (Hoelzel *et al.* 1998*a*; Natoli *et al.* 2004), not always correlated to geographical distance. For a highly mobile

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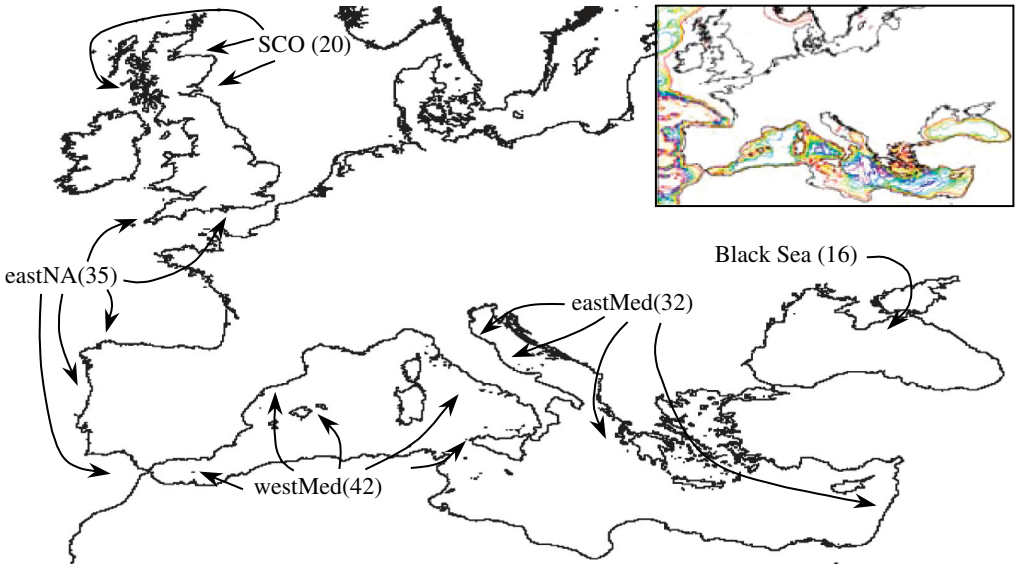

Figure 1. Map of the sample locations. Abbreviations are as follows: eastMed, eastern Mediterranean; westMed, western Mediterranean; eastNA, eastern North Atlantic; SCO, Scotland. At the top right, a map showing the depth profile is shown. Bracketed figures are sample sizes.

species, able to migrate for long distances (including distances of up to 1000 km; Wood 1998; Wells *et al.* 1999), these genetic data have shown greater structure than might be expected. However, various studies have shown evidence for a relationship between habitat type and group strategies for resource exploitation in regional populations, which could promote philopatry. For example, bottlenose dolphins in the Moray Firth, Scotland showed a clear relationship between feeding behaviours and submarine habitat characteristics (Hastie *et al.* 2004). Bottlenose dolphins feeding in coastal habitat in the eastern North Atlantic also showed variation in diet according to habitat type (Gannon & Waples 2004), and dependence on specific habitat types such as estuaries and seagrass, which also correlated with group size and cohesion during foraging (Barros & Wells 1998; Allen *et al.* 2001; Gannon & Waples 2004). The disposition of prey (shallow or deep in the water column, clumped or dispersed, etc.) and the habitat type have also been shown to affect group behaviour in this species (Acevedo-Gutierrez & Parker 2000), and individual social groups show preference for specific resources (e.g. Chilvers & Corkeron 2001).

Our hypothesis is that the capacity to adapt to local environments combined with the likely social facilitation of resource exploitation (e.g. Mann & Smuts 1999) have led to the regional population structure seen in coastal populations of this species, and that this may also explain the evolution of population structure in similar species. Here we compare contiguous populations across a geographical range that represents large- and fine-scale habitat structure, and assess the patterns of gene flow in this context.

2. MATERIAL AND METHODS

(a) *Sample collection and DNA extraction*

Samples were collected from stranded animals, by biopsy sampling or scrub sampling (sloughing skin collected on plastic scrub pads). The biopsy system used is as described in Barrett-Lennard *et al.* (1996). DNA was extracted from tissue samples preserved in salt saturated 20% DMSO by a standard phenol/chloroform extraction method (Hoelzel 1998). The distribution of stranded samples suggests a representative population sample.

A total of 145 samples were included (figure 1). Out of these, 81 samples were analysed in this study for the first time (16 from the northern Black Sea off Crimea and Kerch Strait, 2 from the Ionian Sea, 3 from the eastern north Adriatic, 26 from Spain, 5 from the Balearic Islands, 11 from Portugal, and 18 from Galicia), and they were compared with previously analysed samples (3 from Israel, 7 from the Ionian sea, 8 from eastern north Adriatic, 9 from the western Adriatic Sea, 10 from the Tyrrenian Sea, 1 from Algeria, 6 from South England and 20 from Scotland; (Natoli *et al.* 2004)) for the same loci.

(b) *Habitat characteristics*

Data on habitat characteristics across the study range with respect to salinity and sea temperature were taken from the Mediterranean Ocean Data Base (MODB; http://modb.oce.ulg.ac.be/modb/welcome.html#) and the National Oceanographic Data Center (NODC; http://www.nodc.noaa.gov/). Data on chlorophil production were taken from the NASA Sea-viewing Wide Field-of-view Sensor database (SeaWiFS; http://seawifs.gsfc.nasa.gov/).

(c) *Sex determination*

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

(d) *Microsatellite analysis*

Samples were genotyped at nine microsatellite loci: KWM1b, KWM2a, KWM2b, KWM9b, KWM12a derived from *Orcinus orca* (Hoelzel *et al.* 1998*b*), EV37Mn from *Megaptera novaeangliae* (Valsecchi & Amos 1996), TexVet5, TexVet7 and D08 from *T. truncatus* (Rooney *et al.* 1999; Shinohara *et al.* 1997). Polymerase chain reaction (PCR) conditions were as reported in Natoli *et al.* (2004). Amplified DNA was analysed for length variation on 6% polyacrilamide denaturing gels using fluorescent imaging on an automated ABI PRISM 377

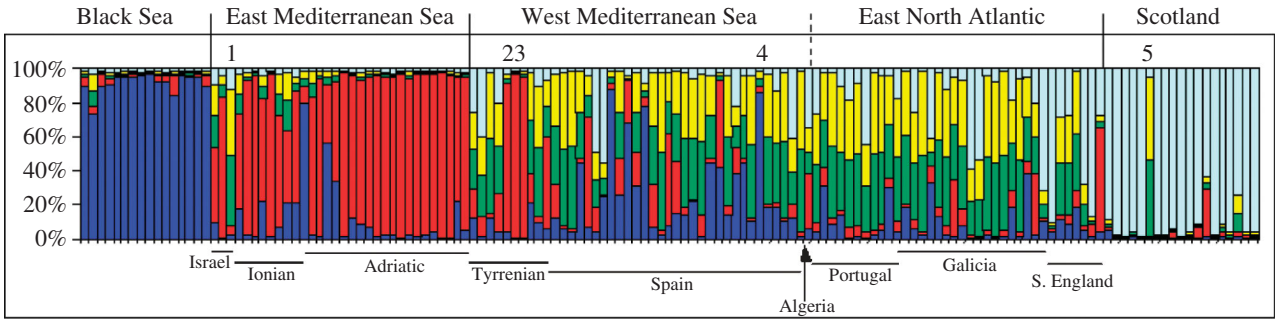

Figure 2. Estimated proportions of the coefficient of admixture of each individual's genome that originated from population K, for $K=5$. Each individual is represented by a column. Detailed geographical origin of the samples is given below the graphic. The numbers 1–5 indicate the individuals identified as migrants (table 3).

DNA sequencer, after incorporation of 1/10 fluorescent labelled primer. An internal standard marker (Genescan-500 ROX, Applied Biosystems) was used to determine the allele sizes.

The level of genetic diversity was estimated as 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 v. 2.9.3 (Goudet 2001). Evaluation of possible deviations from Hardy–Weinberg (HW) equilibrium (overall deviation, heterozygote deficiency and heterozygote excess) was 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) using GENEPOP v. 3.1d (Raymond & Rousset 1995*a*,*b*).

The most probable number of putative populations (K) that best explains the pattern of genetic variability was estimated using the program STRUCTURE v. 1.0 (Pritchard *et al.* 2000). We assumed the admixture model and performed the analysis considering both independent and correlated allele frequency models. Burn-in length and length of simulation were set at 1 000 000 repetitions. To test the convergence of the priors and the appropriateness of the chosen burn-in length and simulation length, we ran a series of independent runs for each value of K (for 1–8) 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 v (migration rate) = 0.05 and 0.1, and testing for G (number of generations) = 0 and 1.

An asymmetric estimate of the migration rate ($M = 4\,N_e\,m$) between pairwise populations, based on microsatellite and mitochondrial DNA (mtDNA) data, was calculated using MIGRATE v. 1.7.3 (Beerli 2002). The lengths of the runs were 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 parameters estimated from the first run and lengthening the Monte Carlo Markov chains. In order to verify the result, a final run was set using longer chains. For comparison with the results from MIGRATE, the migration rate was also calculated from F_{ST} according to $F_{ST} = 1/(4\,Nm + 1)$ (Wright 1951).

Genetic distances between individuals from sample populations were estimated using Nei's Da genetic distance (Nei *et al.* 1983). Calculations were performed using the program Microsatellite Analyser (Dieringer & Schlötterer 2002). Multidimensional scaling analysis was performed using the program XL-STAT PRO v. 6.0 based on a matrix of pairwise Da distances among individuals.

The level of differentiation among populations was estimated as F_{ST} (Weir & Cockerham 1984) using the program ARLEQUIN v. 2.0 (Schneider *et al.* 1999). Sex-biased dispersal was tested using the program FSTAT v. 2.9.3 to compare sex-specific assignment indices and F_{ST} values (for further details, see Goudet 2001). Only adult individuals (a total of 131: 61 females and 70 males) were considered for this analysis.

Evidence for a bottleneck was tested using the programme BOTTLENECK v. 1.2.02 (Cornuet & Luikart 1996). The two-phase model of mutation (TPM) was considered as suggested by the authors (variance for TPM as set equal to 30, proportion of stepwise mutation model in the TPM was set equal to 70%, 1000 iterations).

(e) *mtDNA analysis*

A total of 99 samples were sequenced for 630 bp at the 5′ end of the mtDNA control region (15 from Black sea, 18 from eastern Mediterranean, 31 from western Mediterranean, 35 from the eastern North Atlantic; a geographically representative sample from each region) and compared with 24 sequences already published (1 from Black Sea, 10 from eastern Mediterranean, 4 from western Mediterranean, 9 from Scotland; Natoli *et al.* 2004; accession numbers AY962607–AY962619).

The mtDNA control region was amplified with universal primers MTCRf (5′-TTC CCC GGT GTA AACC) and MTCRr (5′-ATT TTC AGT GTC TTG CTT T) after Hoelzel (1998). The PCR reaction conditions and PCR cycling profile were as reported in Natoli *et al.* (2004). PCR products were purified with QIAgen PCR purification columns and sequenced directly using the ABI dye-terminator method. Sequence alignment was performed using SEQUENCHER v. 3.0 (Gene Code Corporation).

The degree of differentiation (F_{ST}), the nucleotide diversity (π), gene diversity, Tajima's D and Fu's F_S were estimated using ARLEQUIN v. 2.0 (Schneider *et al.* 1999). The Mantel test to estimate the level of correlation between matrices (both to compare mtDNA and microsatellite DNA data and to compare geographical with genetic distance) was also performed using ARLEQUIN v. 2.0. A median-joining network was generated to infer phylogenetic relationships among the mtDNA haplotypes, using the program NETWORK v. 2.0 (Bandelt *et al.* 1999; www.fluxus-engineering.com).

Table 1. Genetic variation at each microsatellite locus for each population.
(The numbers of individuals analysed for each population are indicated below the population names. The number of different alleles, number of private alleles (in parenthesis) and allelic richness (All. Rich.), heterozygosity observed (H_o), and heterozygosity expected (H_e) are reported. The respective averages (standard deviation in parenthesis) are reported in the last rows. The asterisk indicates the loci that showed significant deviation from the Hardy–Weinberg equilibrium (*$p<0.05$). Abbreviations are as in figure 1.)

microsatellites		populations				
		Black Sea ($n=16$)	eastMed ($n=32$)	westMed ($n=42$)	eastNA ($n=35$)	Scotland ($n=20$)
KWM1b	All. Rich.	2, 2	3, (1), 1.988	2, 1.998	2, 1.822	2, 1.943
	H_o	0.375	0.031*	0.286	0.114	0.05
	H_e	0.314	0.122	0.331	0.136	0.189
KWM2a	All. Rich.	3, 3	5, 4.914	6, 4.646	6, 5.582	4, 3.593
	H_o	0.563	0.75	0.524	0.571*	0.75*
	H_e	0.675	0.756	0.605	0.674	0.639
KWM2b	All. Rich.	2, 1.75	4, 2.601	4, 3.166	5, 4.471	3, 2.943
	H_o	0.063	0.156	0.341	0.371*	0.45
	H_e	0.123	0.179	0.406	0.581	0.522
KWM9b	All. Rich.	3, 2.965	4, 3.341	5, (1), 4.064	4, 3.933	4, (1), 3.2
	H_o	0.4	0.419	0.525*	0.618	0.45
	H_e	0.402	0.48	0.68	0.667	0.582
KWM12a	All. Rich.	5, 4.737	7, (1), 5.807	11, 8.212	11, (1), 9.083	7, 5.399
	H_o	0.56	0.69	0.895	0.853	0.65
	H_e	0.677	0.734	0.863	0.896	0.68
EV37Mn	All. Rich.	7, 7	15, (2), 11.781	23, (3), 13.241	19, (1), 12.834	9, 7.22
	H_o	0.5	1	0.949	0.906	0.6
	H_e	0.562	0.928	0.929	0.926	0.778
TexVet5	All. Rich.	4, 3.444	5, 4.662	8, 5.617	9, 6.86	5, 4.303
	H_o	0.25	0.533	0.568	0.552	0.722
	H_e	0.236	0.664	0.651	0.739	0.619
TexVet7	All. Rich.	4, 3.887	4, 3.242	7, (1), 4.375	6, (1), 5.089	4, 3.2
	H_o	0.652	0.194*	0.595	0.543*	0.65
	H_e	0.53	0.341	0.592	0.68	0.53
D08	All. Rich.	4, 3.965	6, 5.448	6, 5.742	8, (2), 6.192	4, 3.833
	H_o	0.6	0.688	0.829	0.647*	0.5
	H_e	0.66	0.737	0.786	0.73	0.489
average (s.d.)	All. Rich.	3.639 (1.574)	4.865 (2.904)	5.673 (3.333)	6.207 (3.195)	3.959 (1.550)
	H_o	0.464 (0.205)	0.549 (0.282)	0.649 (0.197)	0.670 (0.228)	0.557 (0.164)
	H_e	0.438 (0.186)	0.496 (0.321)	0.612 (0.234)	0.575 (0.237)	0.536 (0.213)

3. RESULTS

(a) *Measures of diversity*

Heterozygosity and allelic diversity for all nine microsatellite DNA loci are shown in table 1. Deviation from HW equilibrium for $p<0.05$ was detected in a number of cases (see table 1). This can imply allelic dropout or Wahlund effect. However, if the Bonferroni correction was applied (new $p=0.0011$), no significant deviation was observed. Average allelic richness was lowest for the Black Sea population (3.639) and highest for the east North Atlantic population (6.207). Private alleles were found in all populations except for the Black Sea.

For the 630 bp mtDNA control region sequence, 44 polymorphic sites (7%) were observed identifying a total of 41 different haplotypes (accession numbers AY963588–AY963626). Forty-two transitions, four transversions and two indels were observed. The total average nucleotide diversity was 0.016. The Black Sea population showed the lowest gene diversity (0.675), and the western Mediterranean the highest (0.939), whereas the Scottish population showed the lowest nucleotide diversity (0.006) and the eastern Mediterranean the highest (0.015).

(b) *Inferring population structure*

In order to test the presence of population structure among our samples, we used Structure (Pritchard *et al.* 2000) to estimate the number of populations (K) that best explained the observed genetic variability. 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.

$K=5$ was found to be associated with the highest probability of $P(X/K)$ considering either independent allele frequency or correlated allele frequency models suggesting subdivision into five populations (for example, for correlated data the likelihood is -3473.9 for $K=4$, -3423 for $K=5$, and -3434.4 for $K=6$; figure 2). The clustering identified three clear populations: a Black Sea population, an eastern Mediterranean population including samples from Israel, Ionian Sea and Adriatic Sea, and a Scottish population (figure 2). Two other putative populations: the western Mediterranean (Tyrrenian Sea, Spain, Algeria) and the contiguous eastern North Atlantic were less clearly defined (figure 2). In fact, the two clusters that explained most of the variability of the samples from these two regions (the third,

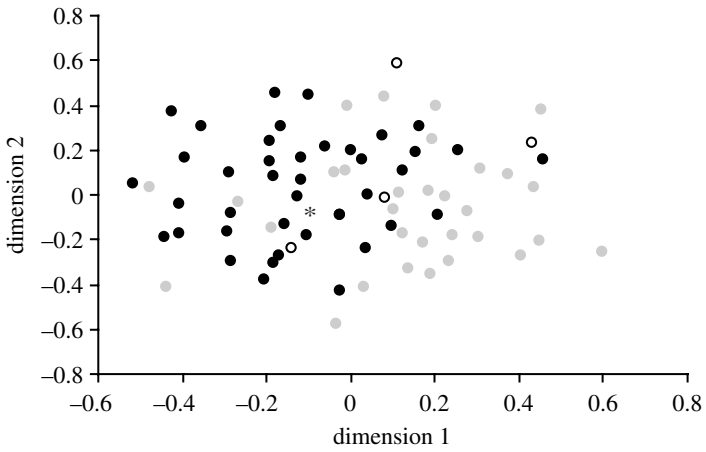

Figure 3. Multidimensional scaling analysis based on D_A distance between pairwise individuals. Symbols represent individuals from the western Mediterranean (black), the eastern North Atlantic (grey), Cadiz (○) and Algeria (*).

Table 2. Pairwise population differentiation values expressed as F_{ST} based on microsatellite data (below diagonal) and mtDNA haplotype frequencies (above diagonal).
(Sample sizes for the microsatellites for each population are reported in the second column. Sample sizes for the mtDNA data are reported in the second row. Statistical significance is reported as follows: *$p<0.05$, **$p<0.01$, ***$p<0.001$. Abbreviations are as in figure 1.)

N. haplo	*N*	Black Sea	eastMed	westMed	eastNA	Scotland
		16	27	35	35	9
Black Sea	16	—	0.041	0.093***	0.140***	0.317***
eastMed	32	0.120***	—	0.032**	0.058***	0.186***
westMed	42	0.102***	0.045***	—	0.040**	0.153***
eastNA	35	0.139***	0.081***	0.026***	—	0.076*
Scotland	20	0.211***	0.152***	0.097***	0.068***	—

q_3, and the fourth, q_4,—shown in figure 2 in yellow and green, respectively) showed low allele frequency divergence (0.03). Therefore, in order to further assess possible population structure between the western Mediterranean Sea and the eastern North Atlantic, the number of populations (K) was estimated considering only the individuals from these regions, as suggested by Pritchard *et al.* (2000). However, no population structure was detected (the most probable number of populations found was for $K=1$). We then assessed this again using multidimensional scaling analysis, based on a D_A distance matrix among pairs of individuals. Clustering was observed consistent with subdivision between the individuals from the western Mediterranean and the eastern North Atlantic (figure 3). Stress was 0.602 in dimension 1, and 0.370 in dimension 2.

Returning to this region in the analysis in STRUCTURE where $K=5$, for each individual the admixture coefficients relative to the third and the fourth group were summed (q_3+q_4). The difference between the average of the individual sums (q_3+q_4) for the western Mediterranean individuals and eastern North Atlantic individuals was found to be significant (Mann–Whitney U-test, $Z=1.962$, $p=0.024$) indicating that the proportion of the ancestry coefficients (q_3+q_4) is different in the two groups of individuals considered.

Deviation from HW equilibrium was tested for the pooled western Mediterranean/eastern North Atlantic group and significant deviation ($p<0.05$) was observed at four loci (one locus if Bonferroni correction was applied ($p<0.001$ 4)).

Differentiation among the five putative populations (Black Sea, eastern Mediterranean, western Mediterranean, eastern North Atlantic and Scotland) at the microsatellite DNA loci was estimated as F_{ST} (table 2). All populations were differentiated ($p<0.001$). The Black Sea population showed the highest level of differentiation when compared with all other populations. The western Mediterranean population was also differentiated from the eastern North Atlantic population, supporting the population subdivision suggested above.

For the mtDNA sequences, population differentiation was estimated as F_{ST} (table 2). All pairwise population comparisons showed significant differentiation except for the Black Sea population compared with the eastern Mediterranean population ($p=0.058$). Significant correlation was found between the mtDNA and microsatellite DNA F_{ST} matrices (Mantel test, d.f.$=4$, $p=0.02$).

A median-joining network was drawn among the different mtDNA haplotypes to visualize the phylogenetic relationship (figure 4), identifying three main clusters. The Black Sea population is represented only in two clusters and all of its unique haplotypes differ by one mutation step from the haplotype shared with the other populations. A similar situation is observed for the eastern Mediterranean population although it is represented in all three clusters. Given the low level of variation observed,

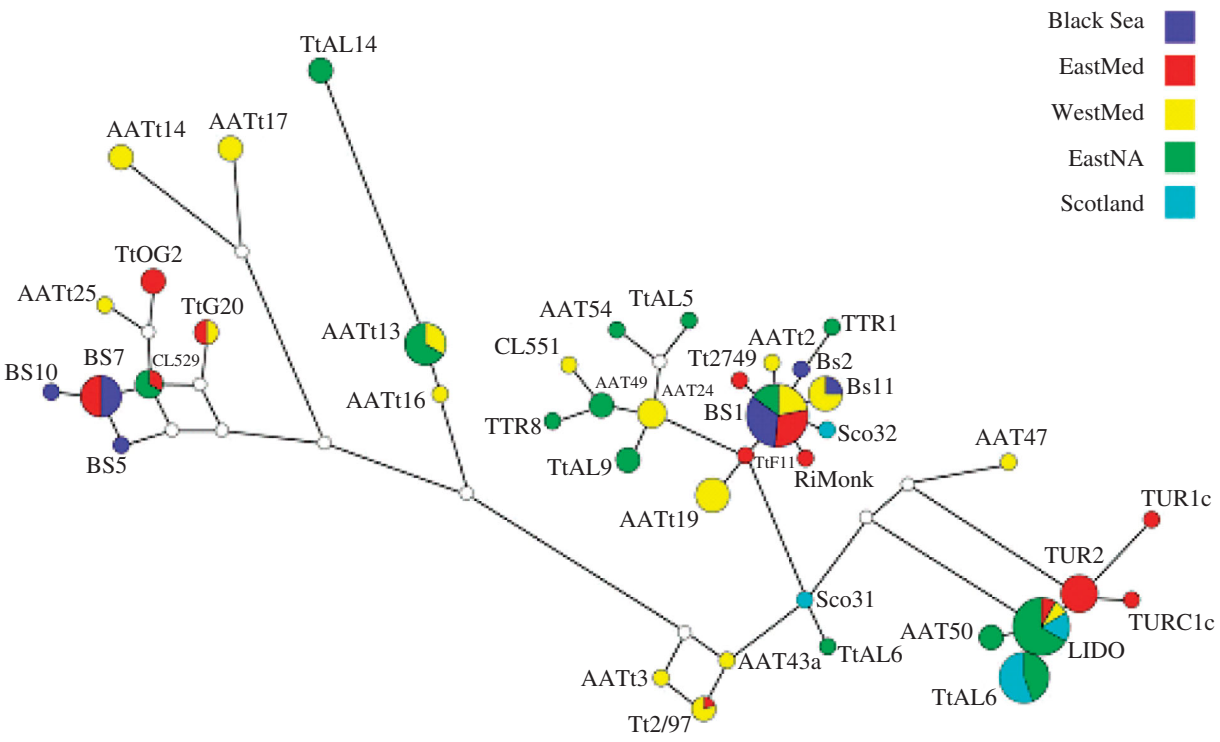

Figure 4. Minimum spanning network among haplotypes. The size of the circles is proportional to the total number of haplotypes observed. Sectors are proportional to the numbers of each haplotype observed in each population. White circles indicate either extinct or unsampled haplotypes.

Table 3. Estimate of the migration rate (M) between contiguous populations based on the microsatellite and mtDNA data. (Nm columns refer to the values calculated according $F_{ST}=1/(4\,N_em+1)$. 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 are as in figure 1.)

population	microsatellites (bi-parental)				mtDNA (maternally inherited)			
	Nm	1,2	2,1	95% CI	Nm	1,2	2,1	95% CI
1 Black Sea	1.830	—	3.490	3.065–3.986	11.690	—	0.046	0.04–0.415
2 eastMed		3.076	—	2.714–3.47		13.304	—	3.986–53.572
1 eastMed	5.306	—	7.813	7.331–8.326	15.125	—	0.558	0.495–2.322
2 westMed		12.857	—	11.935–13.837		10.074	—	4.711–20.845
1 westMed	9.365	—	13.610	12.87–14.382	12.000	—	4.560	4.524–12.608
2 eastNA		16.630	—	15.691–17.652		5.349	—	1.092–5.816
1 eastNA	3.426	—	6.791	6.17–7.45	6.079	—	20.373	13.719–26.546
2 Scotland		4.892	—	4.427–5.376		3.036	—	2.010–4.253

the Black Sea sample was tested for evidence of a population bottleneck using the program BOTTLENECK, and no significant pattern was found. None of the populations had significant Tajima's D-values, and the highest Fu's Fs was 3.28 for the Black Sea.

Mantel tests showed significant correlations between geographical and genetic distance for both mtDNA ($p=0.007$) and microsatellite DNA loci ($p=0.007$).

(c) *Estimating migrants and sex-biased dispersal*

We analysed whether individuals were possible immigrants or descendants of recent immigrants. Because no estimation of the coefficient of migration for the bottlenose dolphin was available in the literature, the analysis was performed three times setting v to 0.001, 0.05 and 0.1 (as suggested in Pritchard *et al.* 2000), and considering four populations (pooling the western Mediterranean and the eastern North Atlantic for this analysis). Five possible immigrant individuals were identified (four males and one female). All individuals were confirmed migrants at the values of $v=0.1$ and $v=0.05$, but none when v was set to 0.001. All individuals had higher probabilities of being immigrants rather than having immigrant ancestry. The Black Sea population did not show any immigrant individual from other areas, while one individual from the western Mediterranean was found to be a possible immigrant from the Black Sea population. In the eastern Mediterranean population, the only immigrant individual detected was one of the three samples from Israel. In the western Mediterranean population two possible immigrants from the eastern Mediterranean population were found. Among the

Scottish samples, one individual was found to be a possible migrant from the western Mediterranean or eastern North Atlantic populations. The ratio of one female to four male dispersers from the assignments in STRUCTURE is not significantly different from equal male and female dispersal (Fisher exact test, $p=0.35$), but the power is very low for such a small sample.

We estimated the migration rate (M) between contiguous populations using two different methods and the results are reported in table 3. The magnitudes of estimates are broadly similar to those provided by estimates based on F_{ST}. The results from MIGRATE for mtDNA show some directional differences not seen in the estimates from microsatellite DNA data. Our theta estimates from MIGRATE range from 0.003 8 to 0.016 6 for the mtDNA data. Simulation studies showed that theta of 0.002 5 and 0.025 gave good estimates of migration rates under similar conditions (see table 3 in Abdo *et al.* 2004). There was no indication of sex-biased dispersal based on the tests undertaken using FSTAT. In fact, no significant heterozygosity deficiency or positive F_{IS} were observed for either sex (F_{IS} for females was 0.079, F_{IS} for males was 0.028, $p=0.18$), and the assignment index was not significant ($p=0.73$, assignment index variance: $p=0.54$). However, this test is known to have relatively low power (see Goudet *et al.* 2002).

4. DISCUSSION

We find clear population structure over the geographical range extending from the Black Sea to Scotland for contiguous populations of the bottlenose dolphin. The putative population boundaries were identified on the basis of comparing individual genotypes in the context of equilibrium expectations with respect to HW and linkage (see Pritchard *et al.* 2000). The result was the assignment of clusters defining boundaries that correspond to physical boundaries in the environment, but none of these is likely to actually restrict the movement of bottlenose dolphins. Instead, they seem to define different habitat regions. Three of the boundaries are relatively strong, suggesting low gene flow, while a fourth is much less well defined.

To take them in turn, the first boundary separates a population in Scotland from samples further south in the North Atlantic. The estimated level of gene flow is relatively low between these two populations, and the mtDNA data suggest a higher rate of female emigration than immigration for the Scottish population. One factor could be geographical distance, as the sample sites are separated by approximately 1200 km. This would be consistent with the results of the Mantel test. However, Scotland is also at the extreme range limit of this species in the North Atlantic. If being at the range limit means that this habitat is marginal (for example with respect to seasonal stress or resource limitations), this would be consistent with the suggested history of female emigration, as mammalian female dispersal behaviour has been shown to be dependent on habitat quality (e.g. Lin & Batzli 2004).

The next boundary divides the North Atlantic samples (collected from Galicia and Portugal) from the western Mediterranean Sea. This is the weakest of the four boundaries, suggesting a relatively high level of continuing gene flow or a very recent division. The Strait of Gibraltar provides a physical boundary, but at a minimum of 10 miles across, it is not one that is likely to restrict the movement of dolphins or their prey. However, the oceanographic feature at the eastern end of the Alboran Sea, the Almeria-Orán front, may serve as a barrier to the movement of some prey species, and perhaps in this way define local populations of their predators. For example, cuttlefish (*S. officinalis*; Perez-Losada *et al.* 2002) and sea bass (*Dicentrarchus labrax*; Naciri *et al.* 1999) both show genetic differentiation either side of this front. If this is the case, it may be recent or a weak mechanism for the isolation of bottlenose dolphin populations, as the data suggest relatively high, bidirectional rates of gene flow across this boundary. Of the five samples collected nearest to the Strait of Gibraltar and Almeria-Orán frontal region, four were near a boundary region in the multi-dimensional scaling plot (figure 3), supporting the possibility that this oceanic front represents the relevant boundary to gene flow.

The next boundary is again stronger, representing the western and eastern basins of the Mediterranean Sea, separated by the Italian peninsula. Genetic differentiation between the eastern and the western Mediterranean has also been observed in other marine species like the common sole (*Solea vulgaris*; Guarniero *et al.* 2002) and the sea bass (*D. labrax*; Bahri-Sfar *et al.* 2000). In those studies the authors suggested that differences in hydrographic characteristics defined the different habitats in these two basins, and promoted the differentiation of intraspecific populations. While the western Mediterranean is more influenced by the Atlantic Ocean, the eastern Mediterranean is characterized by water circulation limited to the Libico-Tunisian Gulf, and by low activity in the rest of the basin (the Adriatic and Aegean Seas), which is under the influence of cool waters of low salinity (Pinardi *et al.* 1997). Again, differences in the distribution of prey, reflecting differences in habitat, may be defining the geographical range and patterns of association in local populations of the bottlenose dolphin. Few details are available on bottlenose dolphin prey choice in the study regions, though primary prey species are known to differ among regions (e.g. hake, *M. merluccius* in the western Mediterranean (Blanco *et al.* 2001) and cod, *Gadus morhua* in Scotland (Santos *et al.* 2001), though in each location, a diversity of prey species was identified).

The final boundary is perhaps the strongest, separating the Mediterranean and Black Seas. Oceanographic conditions change quite dramatically across this boundary, with surface salinity and temperature both very different in the two seas. However, there are also potential historical factors that could lead to population structure, such as a possible founder event when the strait opened, approximately 7800 years ago. Consistent with this is the comparatively low level of diversity found in the Black Sea sample, and the lack of private alleles. However, various tests for evidence of a bottleneck showed no indication of one. This could be due to low power as a consequence of the small sample size, or may instead indicate that the diversity is low because the effective size of the Black Sea population is relatively small. Data from MIGRATE showed the strongest directional effect for gene flow in this population, again suggesting the emigration of females from peripheral (possibly marginal) habitat.

We do not yet know the specific habitat characteristics that may isolate bottlenose dolphin populations, nor do we

know the likely mechanisms. Furthermore, there are certainly finer-scale habitat regions within the broad zones that correlate with genetic structure in this study. However, given division into five genetic populations across a transect from the Black Sea to Scotland, it is striking that the apparent boundaries coincide with regions that can be distinguished for a variety of oceanographic parameters. Based on governmental databases (see §2), the Black Sea shows comparatively uniform and high levels of primary production, low salinity and low surface temperature compared with the eastern Mediterranean. Compared with the western Mediterranean, the east is shallower with greater benthic topographic complexity (figure 1), somewhat lower productivity (SeaWiFS data), and relatively cool waters of low salinity (Pinardi *et al.* 1997). The North Atlantic is much cooler than the Mediterranean, and there is a well-defined thermal boundary that persists throughout the year near the Strait of Gibraltar at the Almeria-Orán front, where we find preliminary evidence for a genetic boundary for bottlenose dolphins, as has been found for various other species (see §1).

Taken together, these data suggest that local populations of bottlenose dolphins are habitat dependent in a way that defines patterns of movement. As reviewed in §1, local populations have been shown to favour specific habitat types (e.g. Barros & Wells 1998; Allen *et al.* 2001; Gannon & Waples 2004). A comparative assessment of estimates of gene flow for mtDNA and bi-parental markers, together with the very similar pattern of F_{ST} values for the two marker types (based on relative magnitude and the Mantel test), indicate that this pattern of movement is true for both sexes (with the exception of differential female movement at range margins). One possible mechanism would be social facilitation of foraging strategies within local communities of dolphins, tending to keep both males and females near their natal site. There are data to support this hypothesis based on apparent group coordination (e.g. Hoese 1971; Janik 2000) and subadult learning (e.g. Mann & Smuts 1999), though more data are needed. Transferable knowledge over generations could be advantageous to assure feeding success; however, it implies complex social structure and long-term individual associations across generations (see Whitehead 1998). This could lead to fine-scale structure at the intra-specific level, and could possibly lead to relatively frequent speciation within the genus (see Natoli *et al.* 2004). However, as indicated by the mtDNA spanning network data, structuring across the study range is probably quite recent, as there is no indication of lineage sorting.

A possible alternative interpretation would be that differentiated populations represent divergence in allopatry followed by the more recent convergence of populations in parapatry. Data on the geological history of the Mediterranean region (e.g. Scotese *et al.* 1998) suggest land mass barriers between the North Atlantic, the western and eastern Mediterranean, and the region that was to become the Black Sea into the late Miocene (5–10 Myr ago). These basins were quite open in the Eocene, approximately 40 Myr ago. Since the oldest delphinid cetaceans date to the Miocene (possibly 11 Myr ago; Barnes 1990), the colonization of this region by dolphins must have been more recent, from the North Atlantic eastward. Therefore, a progressive colonization from the west seems more probable than isolation in allopatry and subsequent reconvergence. The lack of lineage sorting is also inconsistent with divergence in allopatry.

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REFERENCES

Abdo, Z., Crandall, K. A. & Joyce, P. 2004 Evaluating the performance of likelihood methods for detecting population structure and migration. *Mol. Ecol.* **13**, 837–851.

Acevedo-Gutierrez, A. & Parker, N. 2000 Surface behavior of bottlenose dolphins is related to spatial arrangement of prey. *Mar. Mamm. Sci.* **16**, 287–298.

Allen, M. C., Reid, A. J., Gaudet, J. & Sayigh, L. S. 2001 Fine-scale habitat selection of foraging bottlenose dolphins *Tursiops truncatus* near Clearwater, Florida. *Mar. Ecol. Prog. Ser.* **222**, 253–264.

Bahri-Sfar, L., Lemaire, C., Ben Hassine, O. K. & Bonhomme, F. 2000 Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. *Proc. R. Soc. B* **267**, 929–935.

Bandelt, H.-J., Foster, P. & Röhl, A. 1999 Median joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**, 37–48.

Barnes, L. G. 1990 The fossil record and evolutionary relationships of the genus *Tursiops*. In *The bottlenose dolphin* (ed. S. Leatherwood & R. R. Reeves), pp. 3–26. San Diego: Academic Press.

Barrett-Lennard, L. G., Smith, T. G. & Ellis, G. M. 1996 A cetacean biopsy system using lightweight pneumatic darts, and its effect on the behavior of killer whales. *Mar. Mamm. Sci.* **12**, 14–27.

Barros, N. B. & Wells, R. S. 1998 Prey and feeding patterns of resident bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. *J. Mammal.* **79**, 1045–1059.

Beerli, P. 2002. MIGRATE: documentation and program, part of LAMARC. Version 1.7.3. Revised August 7, 2002. http://evolution.genetics.washington.edu/lamarc.html.

Bérube, M. & Palsbøll, P. 1996 Identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers. *Mol. Ecol.* **5**, 283–287.

Bérubé, M., Aguilar, A., Dendanto, D., Larsen, F., Notarbartolo di Sciara, G., Sears, R., Sigurjonsson, J., Urban-R. J. & Palsboll, P. J. 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. *Mol. Ecol.* **7**, 585–599.

Blanco, C., Salomon, O. & Raga, J. A. 2001 Diet of the bottlenose dolphin (*Tursiops truncatus*) in the western Mediterranean Sea. *J. Mar. Biol. Assoc. UK* **81**, 1053–1058.

Chikhi, L., Agnese, J. F. & Bonhomme, F. 1997 Strong differences of mitochondrial DNA between Mediterranean Sea and Eastern Atlantic populations of *Sardinella aurita*. *C. R. Acad. Sci. III* **320**, 289–297.

Chilvers, B. L. & Corkeron, P. J. 2001 Trawling and bottlenose dolphin's social structure. *Proc. R. Soc. B* **268**, 1901–1905.

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.

Dieringer, D. & Schlötterer, C. 2002 Microsatellite analyzer (MSA)—a platform independent analysis tool for large microsatellite data sets. *Mol. Ecol. Notes* **3**, 167–169.

Gannon, D. P. & Waples, D. M. 2004 Diets of coastal bottlenose dolphins from the US mid-Atlantic coast differ by habitat. *Mar. Mamm. Sci.* **20**, 527–545.

Garcia-Martinez, J., Moya, A., Raga, J. A. & Latorre, A. 1999 Genetic differentiation in striped dolphin *Stenella coeruleoalba* from European waters according to mitochondrial DNA (mtDNA) restriction analysis. *Mol. Ecol.* **8**, 1069–1073.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Goudet, J., Perrin, N. & Waser, P. 2002 Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Mol. Ecol.* **11**, 1103–1114.

Guarniero, I., Franzellitti, S., Ungaro, N., Tommasini, S., Piccinetti, C. & Tinti, F. 2002 Control region haplotype variation in the central Mediterranean common sole indicates geographical isolation and population structuring in Italian stocks. *J. Fish Biol.* **60**, 1459–1474.

Hastie, G. D., Wilson, B., Wilson, L. J., Parsons, K. M. & Thompson, P. M. 2004 Functional mechanisms underlying cetacean distribution patterns: hotspots for bottlenose dolphins are linked to foraging. *Mar. Biol.* **144**, 397–403.

Hershkovitz, P. 1966 A catalogue of living whales. *Bull. US Nat. Mus.* **246**, 1–259.

Hoelzel, A. R. 1998 *Molecular analysis of populations; a practical approach*. Oxford, UK: Oxford University Press.

Hoelzel, A. R., Potter, C. W. & Best, P. B. 1998*a* Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. *Proc. R. Soc. B* **265**, 1177–1183.

Hoelzel, A. R., Dahleim, M. & Stern, S. J. 1998*b* Low genetic variation among killer whales (*Orcinus orca*) in the Eastern North Pacific and genetic differentiation between foraging specialists. *J. Hered.* **89**, 121–128.

Hoese, H. D. 1971 Dolphin feeding out of the water in a salt marsh. *J. Mamm.* **52**, 222–223.

Janik, V. M. 2000 Food-related bray calls in wild bottlenose dolphins (*Tursiops truncatus*). *Proc. R. Soc. B* **267**, 923–927.

Lin, Y. K. & Batzli, G. O. 2004 Movement of voles across habitat boundaries: effects of food and cover. *J. Mammal.* **85**, 216–224.

Mann, J. & Smuts, B. 1999 Behavioral development in wild bottlenose dolphin newborns (*Tursiops sp.*). *Behaviour* **136**, 529–566.

Morozova, N. N. 1981 Contemporary state of Black Sea dolphin populations. *Rybnoye Khozyaystvo* **N4**, 45–46. (In Russian.)

Naciri, M., Lemaire, C., Borsa, P. & Bonhomme, F. 1999 Genetic study of the Atlantic/Mediterranean transition in sea bass (*Dicentrarchus labrax*). *J. Hered.* **90**, 591–596.

Natoli, A., Peddemors, V. M. & Hoelzel, A. R. 2004 Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. *J. Evol. Biol.* **17**, 363–375.

Nei, M., Tajima, F. & Tateno, Y. 1983 Accuracy of estimated distance from a number of individuals. *Genetics* **89**, 538–590.

Notarbartolo di Sciara, G., Venturino, M. C., Zanardelli, M., Bearzi, G., Borsani, J. F. & Cavalloni, B. 1993 Cetaceans in the central Mediterranean Sea: distribution and sighting frequencies. *Bull. Zool.* **60**, 131–138.

Perez-Losada, M., Guerra, A., Carvalho, G. R., Sanjuan, A. & Shaw, P. W. 2002 Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) around the Iberian Peninsula indicated by microsatellite DNA variation. *Heredity* **89**, 417–424.

Pinardi, N., Korres, G., Lascarotos, A., Roussenov, V. & Stanev, E. 1997 Numerical simulation of the interannual variability of the Mediterranean Sea upper ocean circulation. *Geophys. Res. Lett.* **24**, 425–428.

Pritchard, J. K., Stephen, M. & Donnelly, P. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Quesada, H., Zapata, C. & Alverez, G. 1995 A multilocus allozyme discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors. *Mar. Ecol. Prog. Ser.* **11**, 99–115.

Raymond, M. & Rousset, F. 1995*a* An exact test for population differentiation. *Evolution* **49**, 1280–1283.

Raymond, M. & Rousset, F. 1995*b* GENEPOP (version 1.2): population genetic software for exact tests and ecumenicism. *J. Hered.* **86**, 248–249.

Roldàn, M. I., Garcia-Marin, J. L., Utter, F. & Pla, C. 1998 Population genetic structure of European hake, *Merluccius merluccius*. *Heredity* **81**, 327–334.

Rooney, A. P., Merritt, D. B. & Derr, J. N. 1999 Microsatellite diversity in captive bottlenose dolphins (*Tursiops truncatus*). *J. Hered.* **90**, 228–231.

Ryan, W. B. F. *et al*. 1997 An abrupt drowning of the Black Sea shelf. *Mar. Geol.* **138**, 119–126.

Santos, M. B., Pierce, G. J., Reid, R. J., Patterson, I. A. P., Ross, H. M. & Mente, E. 2001 Stomach contents of bottlenose dolphins (*Tursiops truncatus*) in Scottish waters. *J. Mar. Biol. Assoc. UK* **81**, 873–878.

Schneider, S., Roessli, D. & Excoffier, L. 1999 Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* **152**, 1079–1089.

Scotese, C. R. *et al*. 1998 *The paleogeographic atlas of Northern Eurasia*. Moscow, Russia: Institute of Tectonics of Lithospheric Plates, Russian Academy of Sciences. (26 maps)

Shinohara, M., Domingo-Roura, X. & Takenaka, O. 1997 Microsatellite in the bottlenose dolphin *Tursiops truncatus*. *Mol. Ecol.* **6**, 695–696.

Tomilin, A. G. 1957 *Mammals of the USSR and adjacent countries. Cetaceans*, vol. IV. Moscow: USSR Academy of Science Publication House. (In Russian.)

UNEP 1996 *State of the marine and coastal environment in the Mediterranean region. MAP Technical Report Series No. 100*. Athens: UNEP.

Valsecchi, E. & Amos, B. 1996 Microsatellite markers for the study of cetacean populations. *Mol. Ecol.* **5**, 151–156.

Weir, B. S. & Cockerham, C. C. 1984 Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

Wells, R. S., Rhinehart, H. L., Cunningham, P., Whaley, J., Baran, M., Koberna, C. & Costa, D. P. 1999 Long distance offshore movements of bottlenose dolphins. *Mar. Mamm. Sci.* **15**, 1098–1114.

Whitehead, H. 1998 Cultural selection and genetic diversity in matrilinear whales. *Science* **282**, 1708–1711.

Wood, C. J. 1998 Movement of bottlenose dolphins around the south-west coast of Britain. *J. Zool.* **246**, 155–163.

Wright, S. 1951 The genetical structure of populations. *Ann. Eugen.* **15**, 323–354.

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