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Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin

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The existence of nearshore and offshore populations of the bottlenose dolphin has been documented throughout its range. In several cases the two regional forms have been shown to be morphologically distinct, although there is considerable overlap for most characters. The populations off the eastern coast of North America have been the subject of a long-term programme of research on their distribution and movements. In this study, we compare mitochondrial and nuclear genetic markers between dolphins classified as either nearshore or offshore type. These putative populations were found to be distinct at both nuclear and mitochondrial genetic markers. Further, the level of variation among the nearshore dolphins was reduced compared with the offshore population. A broader geographical comparison suggests a shared lineage between offshore dolphins from the western North Atlantic and both offshore and nearshore dolphins from the eastern Atlantic. These results are consistent with local differentiation based on habitat or resource specialization in the western North Atlantic, and suggest differences in the character of the nearshore/offshore distinction in different parts of the world.

Keywords: population genetics; marine mammals; resource specialization

1. INTRODUCTION

The bottlenose dolphin (*Tursiops truncatus*) is a social species with a very wide distribution in cold temperate to tropical waters. There are geographical variations in morphotype that have led some in the past to divide the genus into as many as 20 different species (see Hershkovitz 1966), although many of these were based on very limited data. The eastern North Pacific populations have been divided into an offshore form (*T. nuuanu*; Andrews 1911) and the larger nearshore *T. gilli*. A distinct nearshore species in the South Atlantic and Indian Ocean, *T. aduncus*, was also proposed (Ross 1977), and this latter distinction has recently been supported by two phylogenetic studies based on mitochondrial DNA (mtDNA) markers (Curry 1997; LeDuc 1997). However, there is considerable overlap between some morphological characters for these putative species, including *T. nuuanu* compared with *T. gilli* (Walker 1981) and *T. aduncus* compared with *T. truncatus* (Ross & Cockcroft 1990), and most now recognize a single species, *T. truncatus* (see Mitchell 1975; Ross & Cockcroft 1990; Wilson & Reeder 1993), with the possible exception of *T. aduncus*.

A distinction between nearshore and offshore forms has been described for this species in a number of geographic locations (Ross 1977, 1984; Walker 1981; Duffield *et al.* 1983; Ross & Cockcroft 1990; Van Waerebeek *et al.* 1990; Mead & Potter 1995). Two studies compare similar parameters between nearshore and offshore populations in the eastern North Pacific (ENP) and the western North Atlantic (WNA). Walker (1981) compared several cranial characters and found primarily modal distinctions between the nearshore (*T. gilli*) and offshore (*T. nuuanu*) forms in the ENP. For all measured characters, the nearshore population was relatively larger. Both parasite load and diet further distinguished the two populations, with the nearshore dolphins preying on coastal fish species, especially fish in the families Sciaenidae and Embiotocidae, and the offshore dolphins preying on epipelagic fish species and cephalopods (Walker 1981).

A parallel study in the WNA revealed a similar level of distinction between nearshore and offshore forms (Mead & Potter 1995). Unlike the ENP bottlenose dolphins, the populations in the WNA had not been previously described as different species or subspecies (all had been recognized as *T. truncatus*). Measurements reflecting the size of the dolphins (total length and skull length) showed a modal difference with extensively overlapping distributions and, in contrast to the ENP populations, it was the offshore type that was larger.

One cranial character, the relative width of the internal nares, showed a diagnostic difference between the two WNA forms, with the offshore type having consistently wider nasal bones. As in the ENP populations, there was a clear difference in both parasite load and diet. Stomach content analysis indicated that the nearshore dolphins

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preyed predominantly on coastal species of sciaenid fish and a coastal species of cephalopod (*Loligo* sp.), whereas the offshore dolphins preyed on pelagic species of squid and fish, especially fish in the family Myctophidae (Mead & Potter 1995).

Aduncus-type dolphins occur on the east coast of South Africa as far west as False Bay (Findlay *et al*. 1992). This same form has been recognized in Australia (see Ross & Cockcroft 1990). Populations of larger *truncatus*-type dolphins are found further offshore along South Africa's south-east coasts, and may have a continuous distribution offshore around the whole coast. A nearshore population of the *truncatus* type is also found along the coast of Namibia (Findlay *et al*. 1992). The *aduncus* type is the most distinct of the regional morphotypes, being distinguished by its small size and spotted pigmentation pattern on the ventral surface (among other features, see Ross (1977)).

The main objective of the current study is to compare the nearshore and offshore populations off the north-eastern coast of North America from Florida to Massachusetts using nuclear and mtDNA genetic markers. Both nearshore and offshore populations in this region show a seasonal shift in distribution with most sightings south of Cape Hatteras (North Carolina) in the winter, and extending north as far as Massachusetts in the summer (Mead & Potter 1990). Additional samples from elsewhere in the Atlantic (including South Africa) are included in a broader comparative analysis.

2. MATERIALS AND METHODS

(a) *Sample collection and DNA extraction*

For the study comparing the nearshore and offshore populations from the east coast of North America (the WNA populations), most samples were obtained from stranded dolphins and dolphins caught in nets as bycatch. Strandings were from Tybee Island, Georgia, through to Brigantine, New Jersey, with most from North Carolina, Virginia and Maryland. Of the 29 dolphins classified as nearshore types, 22 were from strandings and seven were taken for captive display or incidentally caught from nearshore populations. Seven of those classified as offshore types were from strandings. A further 19 offshore types were from bycatch at or near the edge of the continental shelf, 100–300 miles from the coasts of Georgia, North Carolina, New Jersey, Massachusetts and Maine. Most samples in each data set were collected over ranges of about 500 miles, which overlapped by about 300 miles, with the offshore samples extending further north. This reflects what is known about the distribution of these two putative stocks (Mead & Potter 1990).

Samples from live capture or bycatch could be classified by the location of the catch. Stomach contents and parasite load are diagnostic when prey or parasites found in only nearshore or offshore habitat are found (see Mead & Potter 1995), and most stranded samples used in this study were classified by one or both of these criteria when collected for the Smithsonian archive. A cranial measure found in an earlier study to classify dolphins into two groups, the relative width of the internal nares (Mead & Potter 1995), was consistent with voucher specimens (as determined by parasites, prey and capture location) and further confirmed by our genetic analyses (see below). A plot of internal nares' width against condylobasal length (overall length of the skull) is shown for all the WNA nearshore and offshore samples included in this genetic study for which intact skulls were available ($n=38$, figure 1), illustrating the distinction between the two types. All classification characteristics were consistent across age and sex classes.

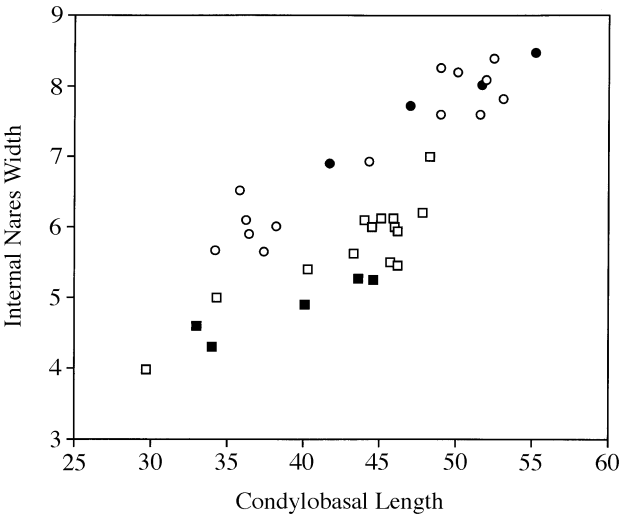

Figure 1. Plot of internal nares' width against condylobasal length (the length of the skull measured from the tip of the rostrum to the occipital condyl) for the samples from the WNA region, showing the distinction between nearshore (squares) and offshore (circles) morphotypes. Samples that could be classified as nearshore or offshore based on some characteristic other than morphology are indicated by open symbols.

Additional samples were obtained from dolphins taken in live capture from a nearshore habitat in the Bahamas ($n=4$ blood samples), from bycatch 60 miles off the coast of Senegal, south of Dakar ($n=2$), from bycatch 300 miles off the coast of El Salvador ($n=1$), and from South Africa and Namibia ($n=21$). The samples from South Africa ($n=17$) were from strandings (from St Helena Bay to Durban) and bycatch (one from a nearshore shark net near Durban and another from 200 miles off the south-eastern coast). The samples from Namibia ($n=4$) were from strandings, two from Walvis Bay, one from Hentjies Bay, and a live capture from the beach at Walvis Bay. South African and Namibian samples were first classified as either *aduncus* type or *truncatus* type based on vertebral count, supported by tooth counts, size and relative rostrum length (see Ross 1977). The *truncatus* types were then classified as nearshore or offshore based on their stomach contents (e.g. mullet being taken as evidence for coastal feeding), parasite load (*Phyllobothrium* sp. being taken as evidence of offshore feeding) and capture location. All positive classifications were based on stomach contents, parasites or both.

DNA was extracted from frozen skin and blood samples by standard phenol/chloroform extraction methods (see Hoelzel 1992). Samples stored as museum preparations in formalin ($n=12$) were finely minced and pre-treated with three washes of distilled water over three days. They were then digested over 5–6 days with Pronase at 37 °C in 50 mM Tris (pH 8.0), 20 mM EDTA (pH 8.0) and 2% SDS, and extracted with phenol/chloroform as above. Note that the differential quality of samples meant that not all samples could be amplified at all loci.

(b) *Microsatellite analysis*

Microsatellite DNA primers for five loci were derived from a DNA library constructed for a killer whale study (Hoelzel *et al*.

Table 1. *Microsatellite allele frequency for each locus for the WNA nearshore (WNAN) and offshore (WNAO) populations*
(Observed (H_O) and expected (H_E) heterozygosities are given for each locus.)

	allele 1	2	3	4	5	6	7	8	9	10	11	12	13	14	H_O (s.e.)	H_E
KWM1b																
WNAN	43	9	0												0.346 (0.093)	0.286
WNAO	1	44	1												0.087 (0.058)	0.084
KWM2a																
WNAN	0	0	0	1	1	0	2	3	1	21	2	15	2	6	0.778 (0.080)	0.751
WNAO	1	1	1	1	3	2	2	1	3	3	4	12	11	1	0.955 (0.043)	0.848
KWM2b																
WNAN	0	48	0	2											0.080 (0.056)	0.077
WNAO	5	36	2	1											0.318 (0.099)	0.315
KWM9b																
WNAN	5	25	19	5	0	0	0	2							0.607 (0.095)	0.668
WNAO	0	2	8	5	10	8	13	0							0.826 (0.079)	0.799
KWM12a																
WNAN	10	0	22	18	4	0	3	0	0	1	0				0.689 (0.085)	0.722
WNAO	3	1	0	4	18	8	4	2	1	0	3				0.619 (0.104)	0.771

1998). The primer sequences are (sense primer given first): locus KWM1b, 5′-TAAGAACCTAAATTTGGC, 5′-TGTTGGGTCTGATAAATG; locus KWM2a, 5′-GCTGTGAAAATTAAATGT, 5′-CACTGTGGACAAATGTAA; locus KWM2b, 5′-AGGGTATAAGTGTTAAGG, 5′-CAACCTTATTTGGATTTC; locus KWM9b, 5′-TGTCACCAGGCAGGACCC, 5′-GGGAGGGGCATGTTTCTG; locus KWM12a, 5′-CCATACAATCCAGCAGTC, 5′-CACTGCAGAATGATGACC.

Amplified DNA was analysed for length variation on a 6% polyacrylamide denaturing gel after incorporation of ^{33}P alpha dATP (PCR reaction conditions: 100 μM dCTP, dTTP and dGTP, 5 μM dATP, 1.5 mM MgCl, 10 mM Tris-HCl, pH 8.4, 50 mM KCl, 250 pM of each primer and 0.1 μCi of ^{33}P alpha dATP). PCR product was denatured at 95 °C for 5 min and chilled on ice for 1 min before loading.

(c) *SSCP analysis and DNA sequencing*

Amplified mtDNA was analysed for single-strand conformational polymorphisms (SSCP; Orita *et al*. 1989). Primers were designed to amplify from the 5′ end of the control region over a range of 300 base pairs (bp) (see Hoelzel *et al*. 1998). PCR product was labelled by incorporation of ^{33}P alpha dATP, as described above for microsatellite analysis. Denatured product was then run on a non-denaturing polyacrylamide gel (37.5:1 acrylamide/bis, 4.5%, 10% glycerol run at room temperature).

Unique SSCP bands were sequenced, including up to six individuals to confirm that unique band mobilities represent unique sequences and that different individuals with the same SSCP genotype had identical sequences. PCR product was sequenced directly using either primers tailed with the universal primer sequences and the ABI dye-primer method, or with standard PCR primers and the ABI dye-termination method.

(d) *Population and phylogenetic analysis*

Possible differences in heterozygosity at microsatellite loci were tested using likelihood ratio tests (in 2×2 contingency tables). The standard error of heterozygosity was estimated using the formulations of Weir (1996). Allele frequency differences at microsatellite loci were investigated using the χ^2 distribution (the χ^2 test was used owing to the occurrence of zeros in a number of allelic classes). Analysis of R_{ST} for microsatellite data (Slatkin 1995) was used to assess population differentiation based on allele frequencies using the MICROSAT computer program (Minch *et al*. 1995). For mtDNA loci, differentiation was assessed using the ϕ_{ST} statistic from the AMOVA computer program (Excoffier *et al*. 1992). Both genetic distance data (per cent nucleotide difference) and haplotype frequency were incorporated into the calculation of the mtDNA ϕ_{ST} statistic. Other measures of diversity (π) and distance (D_{xy}) were computed as described by Nei (1987).

Sequences were compared phylogenetically using the neighbour-joining method (Saitou & Nei 1987) as part of the PHYLIP computer package (Felsenstein 1993). A majority-rule consensus tree was constructed from 1000 bootstrap replications. The ratio of transitions to transversions was set at the observed level of 6:1 (note that one sequence that included an unusual inverted insert is omitted from this calculation; see below). The distance matrix was based on the Kimura two-parameter model (Kimura 1980). The homologous sequence from the killer whale was used as an outgroup.

3. RESULTS

(a) *Population differentiation in the WNA*

Both nuclear and mtDNA markers indicated significant differentiation between the nearshore and offshore forms from the WNA region. Allele frequencies for each of the five microsatellite loci are given in table 1. A significant difference in allele frequency was seen for all five loci, although three loci showed greater differentiation than the other two (table 2). There are alleles unique to one population at each locus, and for most (15 out of a total of 19 unique alleles) these are found in the offshore population. In most cases (11 out of 15), these are relatively rare alleles. At each locus there is greater allelic diversity in the offshore population. Measures of population differentiation (using R_{ST}) vary between loci, but are very high for several loci (table 2). Combining data for all loci gives an R_{ST} of 0.373. Genetic dispersal between the two populations ($Nm = 0.21$) can then be estimated using the

Table 2. *Statistical comparison of allele frequency, H_O and genetic diversity (R_{ST}) between WNA nearshore and offshore populations at five microsatellite loci*

locus	allele frequency χ^2	p	d.f.	heterozygosity G	p	d.f.	R_{ST}
KWM1b	64.1	<0.0001	2	5.05	0.03	1	0.667
KWM2a	31.9	0.0025	13	3.66	0.07	1	0.008
KWM2b	8.70	0.033	3	4.44	0.038	1	0.004
KWM9b	61.7	<0.0001	7	1.90	0.17	1	0.578
KWM12a	58.9	<0.0001	10	0.53	0.47	1	0.377

formulations of Slatkin (1995). Observed heterozygosity is not significantly different from expected values for any of the loci, and the comparison of heterozygosity between populations showed no consistent pattern (table 2).

None of the 18 mtDNA WNA haplotypes were shared between the two populations (figure 2 and table 3). Again, there is greater diversity in the offshore population (12 compared with 6 haplotypes, and $\pi=0.027$ in the offshore population, $\pi=0.006$ in the nearshore population). The average number of nucleotide differences between populations was estimated as $D_{xy} = 0.039$ (after Nei 1987). Another measure of population differentiation (ϕ_{ST}) indicates that 60.4% of the variation can be explained by differences between the two populations. An estimate of the rate of genetic dispersal based on this measure ($Nm=(1/\phi_{ST} - 1)/2$) suggests the dispersal of approximately one female every three generations ($Nm=0.33$).

A haplotype unique to the offshore population includes an unusual insertion of 55 bp, apparently derived from an inversion of the sequence 32–86 bp 3′ of the insertion site. The insertion event also probably involved the deletion of 11 bp in the 3′ flanking sequence (resulting in an alignment gap of 44 bp, and 11 bp with poor homology and a very high proportion of transversions; see figure 2). The inserted sequence is 86% homologous to the original sequence, suggesting a relatively ancient event.

(b) *Differentiation over a wider geographical range*

The mtDNA haplotypes of dolphins from the WNA populations were compared with samples from other geographical regions (see above). Haplotypes are presented in figure 2, and a phylogeny based on these sequences (using the neighbour-joining algorithm) is presented in figure 3. Some of these samples were collected more than 50 miles from shore, including those off Senegal, El Salvador and one off the coast of South Africa. These group with the lineage dominated by the WNA offshore population, as do *truncatus*-type dolphins stranded in South Africa and Namibia, which were classified as either offshore or nearshore based on stomach contents and parasite load. Other samples identified as being from nearshore dolphins in the Bahamas, and some classified as *aduncus* type off South Africa, cluster within the lineage dominated by the six nearshore haplotypes in the WNA population. If only WNA samples and those from the Bahamas are included in the phylogeny, the same lineage relationships are maintained, but the bootstrap support for the nearshore lineage (marked with a cross in figure 3) becomes 41%, and the support for the offshore lineage is marginally increased from 60% to 73%.

The samples from South Africa are relatively homogeneous compared with those from the WNA populations. One haplotype (O, see figure 2 and table 3) is shared between all three putative populations off southern Africa (as well as with one sample from Senegal), and groups with the lineage dominated by WNA offshore samples. This haplotype differs from that from a WNA offshore sample by just one indel (haplotype θ, see figure 2 and table 3). The other of the two haplotypes seen among the six *aduncus*-type dolphins (P) has an absolute difference (% sequence difference) with haplotype O of 4.1%. Haplotype Q was found in both the WNA offshore and in the South African nearshore populations, and has an absolute difference with haplotype O (the most common haplotype among the samples from southern Africa) of 2.0%.

A recent study of phylogenetic relationships among the delphinid cetaceans using mtDNA markers suggested a species-level distinction between *truncatus*- and *aduncus*-type bottlenose dolphins (LeDuc 1997). If we omit the unique *aduncus*-type haplotype (P) and the closely related haplotype (T) from an unidentified South African dolphin, the consequent tree shows the same lineage relationships and very similar bootstrap values (after 1000 replicates), but the root of the nearshore lineage increases in bootstrap support from the current 32% to 54%.

4. DISCUSSION

The habitats used by the nearshore and offshore forms of bottlenose dolphin in the WNA differ in a number of ways including water temperature, depth, prey diversity and prey species composition. The differential use of these habitats may be a consequence of resource specialization based on one or more of these characteristics. Intraspecific variation correlated with habitat use or resource use has been described for a number of delphinid species (for a review, see Hoelzel (1998)).

There is apparently no clear 'nearshore' or 'offshore' morphotype found in all parts of the species range. Our results indicate a clear distinction between the nearshore and offshore forms in the western North Atlantic, but no such distinction between the nearshore and offshore populations off the coast of southern Africa. In fact, haplotypes from all *truncatus*-type samples off Africa grouped in a mtDNA lineage with the WNA offshore population. Further, the dominant haplotype found among southern African samples was shared between nearshore and offshore dolphins and differed from a WNA offshore haplotype by just one indel. This may indicate that the nearshore–offshore separation among the *truncatus*-type dolphins off Africa is relatively recent, or that there is continuing gene flow. The distinction in the WNA may represent local incipient speciation, but further study involving more populations will be needed to resolve the broader questions about taxonomic status. The two WNA populations have similar nuclear genotypes at some loci, and the branches separating the two mtDNA lineages in the phylogeny are relatively

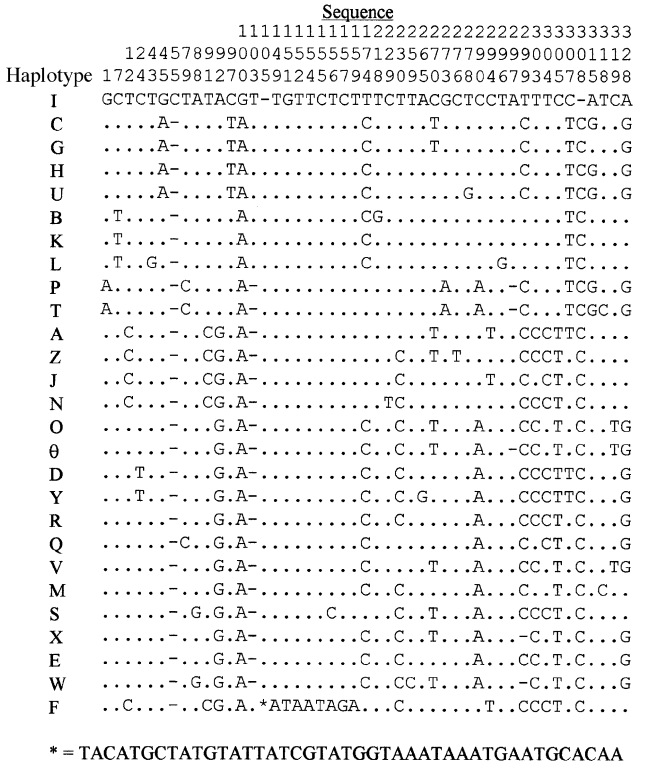

Figure 2. Mitochondrial DNA haplotypes showing the variable sites with reference to haplotype I. The insertion in haplotype F is indicated by '*', and shown at the bottom of the figure. The frequency of each haplotype in each population is given in table 3.

Table 3. *The frequency of mitochondrial DNA haplotypes in each population*

(Population codes: WNAN = western North Atlantic nearshore, WNAO = western North Atlantic offshore, BAH = Bahamas, SAN = southern African nearshore, SAA = southern African *aduncus* type, SAO = southern African offshore, SAU = southern African unknown (both neonates), SEN = Senegal, and ENP = eastern North Pacific.)

	WNAN	WNAO	BAH	SAN	SAA	SAO	SAU	SEN	ENP
haplotype									
I	2								
C	22								
G	1								
H	2								
U	1								
B	1								
K			3						
L			1						
P					4		1		
T							1		
A		8							
Z		1							
J		1							
N								1	
O				3	2	4		1	
θ		1							
D		2							
Y		1							
R						2			
Q		1		1					
V		2							
M									1
S						3			
X		3							
E		2							
W		3							
F		1							

shallow (even when WNA haplotypes are considered in isolation).

The African *aduncus*-type samples were represented by two haplotypes, one grouping with the offshore and the other with the nearshore lineages (although support for the nearshore lineage is strengthened by the removal of this and a closely related haplotype). One haplotype (O) was found in all sample sets from Africa, and a haplotype that differs by only one indel (θ) was found for a dolphin from 40 miles off the north-eastern coast of North America. Our limited data cannot assess the taxonomic status of the aduncus morphotype, but the shared haplotypes do suggest introgression between these local populations, and this is unlikely to be due to the misclassification of samples. The two *aduncus*-type dolphins that shared a haplotype with offshore and nearshore *truncatus*-type dolphins were both sexually mature at a length much shorter than seen in *truncatus*-type dolphins, and were within the *aduncus*-type distributions for all morphological characters. One of these specimens was classified as clearly *aduncus*-type as part of a major study describing the distinction between the two forms (Ross 1977).

Comparisons between the nearshore and offshore WNA populations at both nuclear and mtDNA markers indicated less variation among the nearshore dolphins. Although the nearshore population had six haplotypes, it was dominated by just one (representing 76% of the sample). One possible explanation would be the inclusion of more than one stock in the sample of offshore dolphins. The definition of putative population boundaries in this region is not well established, although patterns of seasonal movement have suggested coherent nearshore and offshore populations along this range (Mead & Potter 1990). There are population genetic data to suggest a stock distinction between dolphins on either side of the Florida peninsula (Dowling & Brown 1992), but not among populations north-east of Florida and south-west of Massachusetts. This would suggest that the unintended inclusion of multiple populations in the sample sets is unlikely, nor is this possibility supported by the molecular data in this study. One possible explanation is that the WNA nearshore population represents a founder event originating from a larger offshore population. The fact that many of the alleles unique to the WNA offshore population are rare would be consistent with this. It may also be that the effective size of the WNA nearshore population is lower owing to other demographic factors. Further, the level of dispersal between populations may be greater for offshore than among the relatively isolated nearshore dolphins. Further data on the migratory and dispersal behaviour of these dolphins would help resolve this question.

The clearest result is the genetic differentiation between the WNA nearshore and offshore populations. This has implications for the management of local stocks and underscores the importance of assessing the genetic structure of delphinid populations that differ in resource

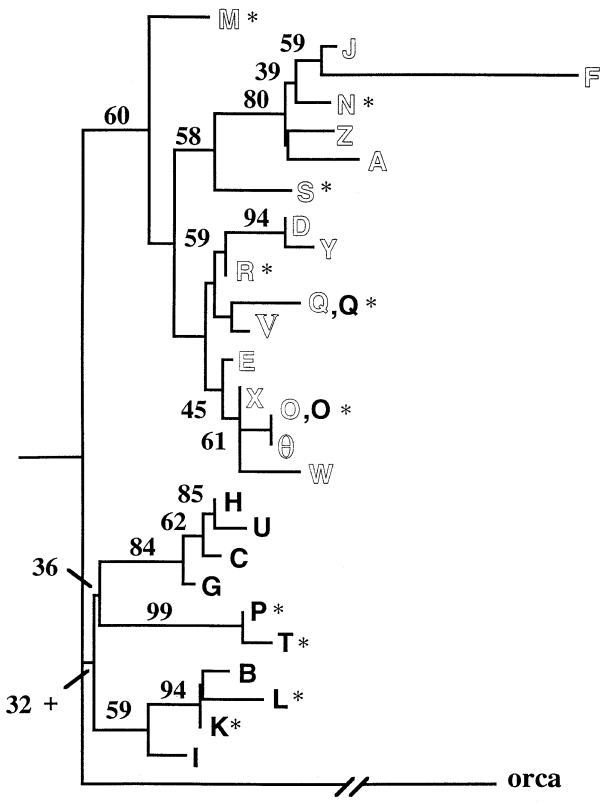

Figure 3. Neighbour-joining tree illustrating the phylogenetic relationships among mtDNA haplotypes (letters correspond to those given in figure 2). Bootstrap values greater than 30 are indicated. Open characters indicate haplotypes from offshore samples, closed characters from nearshore samples (and from both when both are shown). An asterisk indicates that the haplotype is from a population other than (or in addition to) the WNA population. The bootstrap value marked with a '+' increases to 54% when the P and T haplotypes are omitted (see text for discussion).

use either in parapatry or sympatry (see Hoelzel & Dover 1991; Hoelzel *et al*. 1998).

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