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Habitat type promotes rapid and extremely localised genetic differentiation in dolphins

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Abstract. The high potential for dispersal of many marine organisms often results in low population differentiation over large distances. Here, we report that dolphin communities living in very close geographic proximity (<16 km) but in two different environments – open coast and enclosed embayment – exhibit unexpected genetic differentiation at nine microsatellite loci. Results based on a fixation index and a Bayesian clustering approach suggested that gene flow between communities within an embayment is high, as is gene flow between coastal communities. However, lower gene flow between embayment and open coast communities translated into substantial genetic differentiation between dolphin communities from the two environments, and assignment of individuals into two populations. Along with patterns observed in 403 bp of the mitochondrial DNA control region, the results suggest that restriction of gene flow likely occurred in the last 6000 years, after coastal dolphins colonised the embayment. We hypothesise that factors such as fidelity to the local area and resource and behavioural specialisations may have played a major role in promoting and maintaining genetic subdivision between dolphins of the two environments. Importantly, our study shows that habitat type can rapidly promote extremely fine-scale genetic structure in a long-lived, highly mobile marine mammal.

Additional keywords: dispersal, Indo–Pacific bottlenose dolphin, phylogeography, population genetics, *Tursiops aduncus*.

Introduction

The lack of prominent geographical barriers in the marine environment coupled with the high dispersal capabilities of many marine organisms is believed to promote high levels of gene flow and to reduce intra-specific differentiation across large areas (Palumbi 1992). However, recent studies on fishes and marine invertebrates in open ocean regions and around oceanic islands have revealed unpredicted genetic subdivision at very small geographic scales (e.g. Barber *et al.* 2000; Taylor and Hellberg 2003), challenging conventional views on the distribution of biodiversity in the sea (Palumbi and Warner 2003). Genetically divergent marine populations have also been reported for inshore fish species that inhabit environments isolated from ocean currents and characterised by variable environmental conditions, such as enclosed embayments, lagoons and estuaries (e.g. Beheregaray and Sunnucks 2001; Watts and Johnson 2004). These findings demonstrate that the potential for population isolation and genetic differentiation in the marine environment may have been underestimated.

Cetaceans have a wide distribution in the world's oceans and are highly mobile, with individuals from some species migrating or dispersing over extremely large distances, such as between ocean basins, whereas others range over relatively small geographic distances, such as in shallow regions (Hoelzel 1998). Bottlenose dolphins (genus *Tursiops*) are distributed worldwide in temperate and tropical waters and inhabit a wide range of environments, including embayments, open coasts and pelagic waters (Rice 1998). The common bottlenose dolphin *T. truncatus* inhabits both coastal and offshore waters of all oceans (Rice 1998), whereas the Indo–Pacific bottlenose dolphin *T. aduncus* is restricted to coastal and shallow offshore waters of the Indian Ocean, Indo–Pacific Region and the Western Pacific Ocean (e.g. Ross and Cockcroft 1990; Wang *et al.* 1999; Hale *et al.* 2000; Möller and Beheregaray 2001). Bottlenose dolphins living in protected coastal environments (e.g. embayments) usually show a high degree of site fidelity to local areas and belong to relatively small communities or populations (Wells *et al.* 1987), whereas those inhabiting less protected waters (e.g. in open coasts) tend to display more extensive ranging patterns and appear to belong to larger populations (Defran and Weller 1999).

Most cetacean species appear to exhibit a complex pattern of population genetic structure (Hoelzel 1998). For bottlenose dolphins, it has been hypothesised that habitat boundaries and site fidelity in sheltered environments may promote genetic differentiation between dolphin groups, whereas the more expansive ranges of dolphins in open water and on the open coast may

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enhance genetic exchange between adjacent groups (reviewed in Curry and Smith 1997). We tested this hypothesis by analysing the genetic structure of five adjacent communities of Indo–Pacific bottlenose dolphins (*T. aduncus*) from south-eastern Australia that inhabit two different but geographically proximate environments – embayment and open coast. Here we provide evidence for genetic structure on a very small geographic scale (as little as 16 km) in Indo–Pacific bottlenose dolphins and show that this pattern is likely associated with the recent colonisation of an embayment environment.

Material and methods

Study areas and dolphin communities

Port Stephens (PS) (32°42′S, 152°06′E) is a shallow embayment with most depths between 2 m and 8 m. It has ~140 km^2 of surface area, with substrata including seagrass beds, sand and mud flats, rocky reefs and mangrove areas. The eastern Port is a typical marine environment, with ocean tidal influxes, whereas the western Port is typically estuarine, receiving river freshwater outflow. Approximately 90 bottlenose dolphins are sighted in this area on a regular basis (Möller *et al.* 2002), with two socially structured communities – East Port Stephens (EPS) and West Port Stephens (WPS) (Fig. 1) – identified from a long-term photo-identification study. This study started in 1998 and continues to the present date, and covers both breeding (summer) and non breeding (winter) seasons (Möller 2001; Möller *et al.* 2001; Möller *et al.* 2006; J. Wiszniewski, unpub. data). Based on coastal surveys of dolphin distribution and photo-identification realised in the summers and autumns of 2001–2004, three distinct, small coastal communities have also been recognised along the adjacent open coast: Newcastle (NC) (32°55′S, 151°48′E), Broughton Island (BI) (32°36′S, 152°18′E), and Forster (FOR) (32°10′S, 152°32′E) (Fig. 1). This 130 km stretch of coastline between Newcastle and Forster is characterised by sandy beaches interspersed with rocky reefs.

Biopsy samples

Samples from 86 individual dolphins were collected between 1999 and 2004 using a Paxarms (Timaru, New Zealand) biopsy rifle (Krützen *et al.* 2002) and a biopsy pole when dolphins were riding the bow of the boat (Bilgmann *et al.* 2007). Samples from the embayment communities were obtained throughout the year between 1999 and 2000, during surveys carried out in addition to those from the long-term photo-identification study. All samples from the embayment are from known, photo-identified individuals, which belong to either the EPS or WPS communities as determined by range and social network analysis (Möller 2001; Möller *et al.* 2006; J. Wiszniewski, unpub. data). During embayment surveys for biopsy and for photo-identification no coastal dolphins were observed. Samples from the coastal communities were obtained during the same coastal surveys of dolphin distribution and photo-identification (summers and autumns of 2001–2004). The coastal community to which a group of dolphins belonged was determined from the location of sampling, supplemented by photo-identification and visual recognition by personnel with long, detailed experience of the photo-identified individuals (L. Möller and S. Allen). During coastal surveys, no embayment dolphins were observed in the location of coastal communities. Coastal dolphins were re-sighted between years within their community areas, but not between community areas. The only exception is the Newcastle animals, which were sighted once around Broughton Island. Samples collected on that occasion were not included in the study.

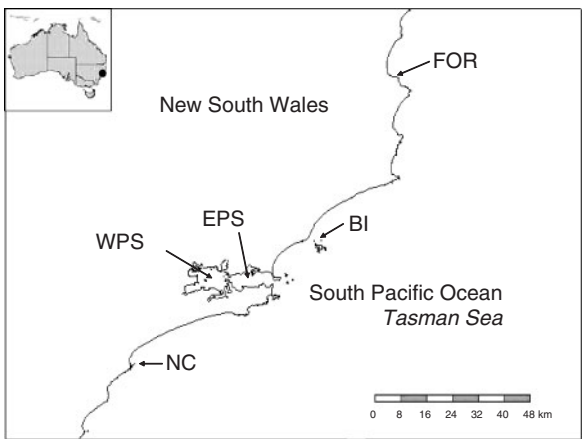

Fig. 1. Study area in south-eastern Australia showing the location of bottlenose dolphin communities. FOR, Forster; BI, Broughton Island; WPS, West Port Stephens; EPS, East Port Stephens; NC, Newcastle.

Genetic methods

DNA was extracted from samples using a salting-out protocol (Sunnucks and Hales 1996) and genetically sexed using fragments of the ZFX and SRY genes (Gilson *et al.* 1998). We used PCR to amplify nine cetacean microsatellites: Ev1 and Ev37 (Valsecchi and Amos 1996), Mk5, Mk6, Mk8 and Mk9 (Krützen *et al.* 2001), D8 (Shinohara *et al.* 1997), Kw2 and Kw12 (Hoelzel *et al.* 1998), using PCR conditions as described in Möller and Beheregaray (2004). We also obtained sequence data of 403 bp of the mtDNA control region, using a protocol described in Möller and Beheregaray (2001). In brief, a fragment of the mtDNA control region was amplified by the polymerase chain reaction (PCR) with primers Dlp-1.5 (5′-TCACCCAAAGCTGRARTTCTA-3′) and Dlp-5 (5′-CCATCGWGATGTCTTATTTAAGRGGAA-3′) from Baker *et al.* (1993). Amplified fragments were screened for sequence variation by the single-stranded conformation polymorphism (SSCP) analysis (Sunnucks *et al.* 2000). Representatives of all identified SSCP phenotypes were then sequenced in an ABI 377 DNA sequencing system (Applied Biosystems, Foster City, USA) according to manufacturer's instructions. The reliability of the technique was confirmed by comparing DNA sequences of several individuals with same and different SSCP phenotypes (as in Sunnucks *et al.* 2000). All individuals with rare phenotypes were sequenced, whereas at least 15% of individuals with common phenotypes were sequenced. All different phenotypes confirmed as different sequences (i.e. haplotypes) and individuals with the same phenotype always had identical sequences.

Microsatellite data analysis

Genetic variation within communities was estimated by calculating mean number of alleles per locus, and expected and observed heterozygosities, using GENEPOP 3.4 (Raymond and Rousset 1995). In addition, allelic richness (AR), a measure that takes

sample size into account, was calculated using FSTAT 2.9.3 (Goudet 2001). Exact test for deviations from Hardy–Weinberg equilibrium and tests for linkage disequilibrium were also carried out in GENEPOP (*P* values obtained with the Markov chain method with 10 000 iterations). Significance levels of all multiple simultaneous comparisons were corrected with the sequential Bonferroni procedure (Rice 1989).

Genetic divergence between pairs of communities was investigated by computing F_{ST} (significance assessed by 10 000 permutations) using ARLEQUIN 2.0 (Schneider *et al*. 2000). Analysis of F_{ST} was preferred over R_{ST} (Slatkin 1995) as the former is more conservative for estimating gene flow when sample sizes are relatively small (Gaggiotti *et al.* 1999), and because the distribution of alleles across several of the loci used (Appendix 1) did not appear to follow a strict stepwise mutation model, on which R_{ST} is based. To test for population genetic structure, a Bayesian model-based clustering method implemented in STRUCTURE 2.1 (Pritchard *et al.* 2000) was used (burn in period of 100 000 iterations, runs of 10^6, values of $K = 1–5$, for each of 5 independent runs). Analyses were performed without and with prior information on the sampling community, with the population admixture model, and with the correlated and with the uncorrelated frequency model. The number of clusters (K) was inferred from the posterior probability distribution Pr (K/X) calculated from the posterior probability of the data Log Pr (X/K).

We reconstructed a tree depicting relationships among communities based on Nei's (1978) genetic distance calculated in BIOSYS 2 (Swofford and Selander 1981). The tree was reconstructed by the UPGMA method with 1000 bootstrap replications using PHYLIP (Felsenstein 1997).

We also used the microsatellite data to conduct assignment tests and relatedness analysis to investigate sex-biased dispersal patterns of coastal dolphins only. The assignment index was computed for each coastal individual with GENECLASS 1.0 (Cornuet *et al*. 1999) using the Bayesian approach of Rannala and Mountain (1997) with the 'leave one out' procedure. Corrected assignment indices (AIc) were then used to determine differences in assignment values between males and females as described in Favre *et al.* (1997). Relatedness estimates were also computed for coastal dolphins only using the index of Queller and Goodnight within RELATEDNESS 5.04 (Goodnight and Queller 1998) (standard errors obtained by jackknifing over all loci). Mean relatedness between males (♂–♂), females (♀–♀), and opposite-sex pairs (♀–♂) were estimated and differences in the mean relatedness between categories were assessed using a two-sample randomisation test within RT 2.1 (Manly 1997). These tests were run to compare with previous data for embayment dolphins reported in Möller and Beheregaray (2004).

Mitochondrial DNA data analysis

Mitochondrial DNA control region sequences were edited and aligned using SEQUENCHER 4.1.2 (Gene Codes Corporation, MI). Genetic variation was estimated in ARLEQUIN 2.0 (Schneider *et al*. 2000) by calculating haplotypic diversity (h) and nucleotide diversity (π) using the Kimura 2-parameter (K2P) genetic distance (Kimura 1980) (with gamma distribution of 0.5). Additionally, allelic richness (AR) was calculated using CONTRIB 1.02 (Petit *et al.* 1998). Genetic divergence between pairs of communities was investigated by computing F_{ST}, which is based on haplotype frequencies (Weir and Cockerham 1984) and Φ_{ST}, which takes haplotype frequencies and molecular distance into account (Excoffier *et al.* 1992) (significance assessed by 10 000 permutations) using ARLEQUIN 2.0 (Schneider *et al*. 2000). A haplotype network was constructed using the maximum-parsimony method of Templeton *et al.* (1992) implemented in TCS 1.06 (Clement *et al.* 2000) to examine the genealogical relationships among mtDNA lineages.

Table 1. Acronym used for each of the five dolphin communities and number of genetically sampled individuals by sex and community

Community	Acronym	Male	Female	Unknown	Total
West Port Stephens	WPS	7	8	0	15
East Port Stephens	EPS	10	10	0	20
Newcastle	NC	9	10	1	20
Broughton Island	BI	14	6	1	21
Forster	FOR	2	8	0	10

Results and discussion

Number of dolphins sampled in each community and sex of individuals are reported in Table 1. Microsatellite loci showed moderate variability in the five communities: mean number of alleles per locus ranged between 3.8 (±0.5) and 5.7 (±0.6) and expected heterozygosities between 0.459 (±0.08) and 0.650 (±0.03) (Table 2). Embayment communities had significantly less genetic variability than coastal communities (Wilcoxon test, $P < 0.05$ for all embayment–coast pairwise comparisons), but no significant differences in variability were observed within embayment and within coastal communities (Wilcoxon test, $P > 0.05$ for all embayment–embayment and coast–coast pairwise comparisons). In addition, coastal communities displayed 21 private alleles compared to only one private allele found in one of the embayment communities at locus MK6 (Appendix 1). All communities were not significantly out of Hardy–Weinberg equilibrium, and there was no evidence for linkage disequilibrium for any locus pair after Bonferroni correction.

Pattern of marked population differentiation between embayment and coastal communities was revealed by both traditional methods (F_{ST} and Nei's genetic distance) and Bayesian clustering. All six pairwise comparisons between embayment and coastal communities resulted in highly significant fixation indices ($P < 0.001$: F_{ST} ranged from 0.06 to 0.15) (Table 3), with F_{ST} values suggesting moderate genetic differentiation (Balloux and Lugon-Moulin 2002) between dolphins of the two environments. These include comparisons between East Port Stephens (embayment) and Broughton Island (coast), two communities less than 16 km apart. In contrast, we found no differentiation among the two embayment communities or among two of the three coastal communities (Newcastle was significantly different from the other coastal communities, although F_{ST} values were low compared to those observed between the coastal and embayment communities) (Table 3). The pattern of genetic structure was confirmed by pooling samples from the embayment ($n = 35$) and coastal communities ($n = 51$). This translated into substantial genetic structure between populations from the

Table 2. Summary of genetic variation within communities of bottlenose dolphins based on nine microsatellite loci and a 403-bp fragment of the mtDNA control region

NA, mean number of alleles per locus; AR, allelic richness; H_E, mean expected heterozygosity; H_O, mean observed heterozygosity; NH, number of haplotypes (standard errors are in parentheses). Acronyms for locations are as in Table 1

Location	Microsatellites				Mitochondrial DNA			
	NA	AR	H_E	H_O	NH	AR	Haplotypic diversity	Nucleotide diversity
WPS	3.8 (0.5)	3.1	0.459 (0.075)	0.491 (0.078)	2	1.0	0.343 (0.128)	0.0026 (0.0021)
EPS	4.2 (0.5)	3.3	0.501 (0.052)	0.552 (0.070)	2	1.0	0.526 (0.040)	0.0040 (0.0028)
NC	5.7 (0.6)	4.1	0.580 (0.053)	0.578 (0.044)	4	2.2	0.552 (0.111)	0.0049 (0.0032)
BI	5.6 (0.4)	4.2	0.662 (0.031)	0.700 (0.051)	3	1.5	0.552 (0.066)	0.0044 (0.0029)
FOR	4.0 (0.4)	3.8	0.650 (0.029)	0.681 (0.044)	3	2.0	0.600 (0.131)	0.0056 (0.0038)

Table 3. Pairwise F_{ST} values among five bottlenose dolphin communities based on nine microsatellite loci (below diagonal) and a 403-bp fragment of the mtDNA control region (above diagonal)

$^*P < 0.05$, $^{**}P < 0.01$

	WPS	EPS	NC	BI	FOR
WPS		0.098	−0.021	0.035	0.381**
EPS	0.012		0.042	−0.040	0.298**
NC	0.083**	0.066**		−0.009	0.209*
BI	0.091**	0.073**	0.018*		0.248**
FOR	0.146**	0.104**	0.030*	0.000	

different environments ($F_{ST} = 0.076$, $P < 0.001$), an outcome also observed when comparing males ($n = 42$) and females ($n = 42$) separately (males $F_{ST} = 0.052$; females $F_{ST} = 0.095$: both $P < 0.01$). In addition negative and non-significant values of F_{IS} were obtained when data from the two embayment communities and from the three coastal communities were combined (data not shown). Graphic representation of Nei's genetic distance based on analyses including all individuals is summarised in an UPGMA tree, which provided strong bootstrap support for the separation of dolphins into two groups (80% for embayment and 75% for coast) (Fig. 2). Although Broughton Island and Forster grouped together separate from Newcastle, support for this grouping was low (<50%). According to STRUCTURE, a large proportion of embayment dolphins (94%) had a higher probability of membership to population 1, while a large proportion of coastal dolphins (86%) showed a higher probability of belonging to population 2 (Fig. 3). Average Q values in these respective populations were 0.77 for dolphins sampled in the embayment and 0.68 for dolphins sampled on the coast. When determining the most likely number of populations in the data set, the highest probability was obtained when K = 2 populations: $P(K/X) = 1$ for K = 2 populations and $P(K/X) = \sim 0$ for K = 1, 3, 4 and 5 populations. Overall, the analyses support the notion that the separation of embayment and coastal communities into two populations is a reliable indicator of the underlying genetic structure.

The two populations also show differences in sex-biased dispersal patterns. While embayment dolphins have been previously shown to have male-biased dispersal tendencies based on genetic data (Möller and Beheregaray 2004), data from this study suggest that coastal dolphins exhibit no sex bias in dispersal. For the

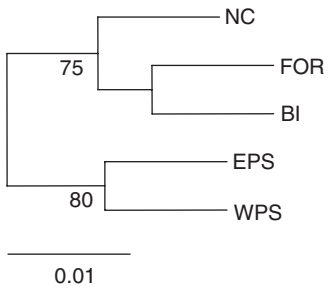

Fig. 2. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendogram based on Nei's (1978) genetic distance for nine microsatellite loci among five bottlenose dolphin communities. Bootstrap values greater than 50% are shown below branches.

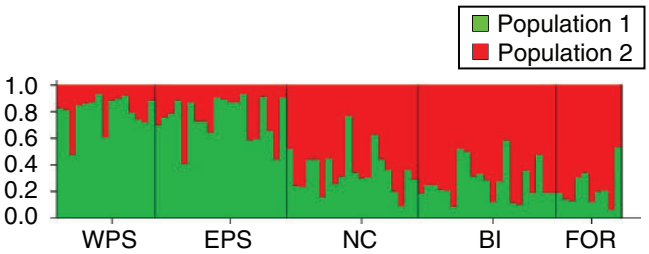

Fig. 3. Bayesian clustering of the five bottlenose dolphin communities based on nine microsatellite loci. Each individual is represented by a vertical column partitioned into two coloured segments, with the length proportional to the individual's estimated membership coefficient in the two populations.

latter, there were no significant differences in mean corrected assignment indices of males and females (♂: −0.32, ♀: −0.03) ($t = 0.43$, $P = 0.673$), nor in mean relatedness between same-sex and opposite-sex comparisons (♂–♂: -0.003 ± 0.015, ♀–♀: -0.028 ± 0.026, ♀–♂: -0.021 ± 0.003) ($P > 0.05$ for all comparisons). In addition, mean corrected assignment indices and mean relatedness were lower for coastal dolphins than for embayment dolphins (Möller and Beheregaray 2004), suggesting higher dispersal overall on the coast. This idea is further corroborated by the F_{ST} values. This result is particularly remarkable because the geographic distance between coastal communities is much greater than the geographic distance between embayment communities.

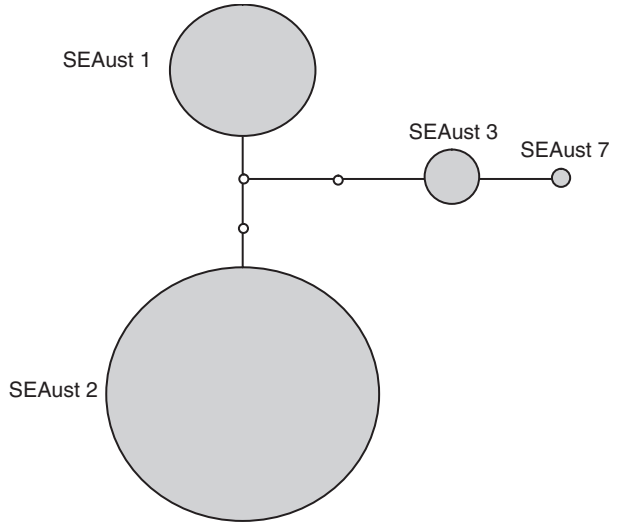

Fig. 4. Haplotype parsimony network depicting genealogical relationships among mtDNA lineages of bottlenose dolphins (haplotype names as deposited in GenBank). The size of ovals is proportional to haplotype frequency. Single lines indicate one mutation between haplotypes and small circles represent missing haplotypes.

Table 4. Distribution of mtDNA haplotypes and total number of samples resolved for each bottlenose dolphin community

Community	Haplotype				Total
	SEAust 1	SEAust 2	SEAust 3	SEAust 7	
WPS	3	12	0	0	15
EPS	9	10	0	0	19
NC	4	13	2	1	20
BI	8	12	1	0	21
FOR	1	3	6	0	10

A historical perspective on the relationships of these dolphin populations was obtained by analysis of the mtDNA control region data. Sequence alignment of a 403-bp fragment of the mtDNA control region revealed four unique haplotypes (Fig. 4, GenBank numbers AF287951–3, EF581128). Similar to nuclear DNA, genetic variation in the mtDNA was lower for embayment dolphins (Table 1). Two abundant haplotypes (SEAust 1 and 2) were present in all communities, whereas two rare haplotypes (SEAust 3 and 7) were found only in coastal communities (Table 4). Similar frequencies of the most abundant haplotypes in East and West PS, Newcastle and Broughton Island (Table 4) resulted in no significant differentiation between the four localities using the fixation index F_{ST} (Table 2) and Φ_{ST} (data not shown). However, due to the relatively high frequency of haplotype SEAust 3 in Forster (Table 4), significant differentiation was observed between this and all other communities, but the high frequency may be an artefact of small sample size from this community.

All mtDNA control region haplotypes appear as closely related in the resulting network (Fig. 4), with maximum absolute sequence divergence of 1.5%. The close genealogical relationship among all haplotypes and the similar frequency of the two abundant haplotypes in both environments provides support for a single population origin of embayment and coastal dolphins. The abundant haplotypes detected in this study are the same high-frequency haplotypes reported for an embayment population located about 400 km further south (Möller and Beheregaray 2001). In addition, the low levels of sequence divergence (0.5–2.2%) between these *T. aduncus* haplotypes from south-eastern Australia and those found in Indonesia, Taiwan and China suggest that these populations of Indo–Pacific bottlenose dolphins have a shallow genealogical history (Möller and Beheregaray 2001). In this regard our results are markedly different from those found in studies of *T. truncatus*, where genetic differentiation between populations at nuclear microsatellite DNA tend to correlate with differentiation at the mtDNA control region (e.g. Natoli *et al.* 2005; Sellas *et al.* 2005). These differences may relate to the fact that, within the subfamily Delphininae, the common bottlenose dolphin *T. truncatus* appears as a more ancient species than the Indo–Pacific species *T. aduncus* (LeDuc *et al.* 1999). Overall, *T. truncatus* are likely to display older population histories, deeper coalescence of mtDNA lineages and associated longer time for geographic sorting of mtDNA haplotypes than *T. aduncus*.

In summary, we found significant differences between embayment and coastal communities of south-eastern Australian Indo–Pacific bottlenose dolphins at microsatellite DNA, with only one private allele in the Port Stephens' embayment against 21 private alleles on the adjacent coast. At the mtDNA control region, however, we generally found no significant genetic differentiation between embayment and coastal communities, with common haplotypes found in both community types and no private haplotypes in the embayment. In combination these data suggest that the pattern of divergence reported here is probably due to a recent colonisation of the embayment by coastal dolphins, followed by a rapid restriction to gene flow. This founder event, which is consistent with a subset of the coastal genetic diversity present in the embayment population, likely occurred during the last 6000 years, after inundation of the Port Stephens' embayment by the last postglacial marine transgression of the Holocene (Roy 1984).

The genetic evidence for restricted gene flow observed over scales of few tens of kilometres in this study (including about 16 km from the entrance of Port Stephens to Broughton Island) is remarkable given the high dispersal capability of bottlenose dolphins. Moderate to high genetic differentiation (Balloux and Lugon-Moulin 2002) between common bottlenose dolphins (*T. truncatus*) at microsatellite DNA has been previously reported for populations inhabiting different habitats but separated by hundreds to thousands of kilometres (e.g. Hoelzel *et al.* 1998; Natoli *et al.* 2005; Sellas *et al.* 2005). Natoli *et al.* (2005) found boundaries of population structure of common bottlenose dolphins from the Black Sea to the eastern North Atlantic to coincide with ocean floor topography and several oceanographic parameters. In the western North Atlantic nearshore and offshore populations of this species, inhabiting waters differentiated by depth, temperature and prey diversity, have also been genetically identified (Hoelzel *et al.* 1998). In the Gulf of Mexico, inshore resident communities of common bottlenose dolphins inhabiting protected bays, sounds and estuaries are genetically differentiated from a coastal population at a similar geographic scale to

that reported here, although only low levels of differentiation were reported (Sellas *et al.* 2005). All these results corroborate the hypothesis put forward by Curry and Smith (1997) that habitat boundaries and residency in sheltered environments may promote genetic differentiation between adjacent dolphin groups.

It has been proposed that evolutionary structuring mechanisms shaping genetic differentiation of common bottlenose dolphin populations may relate to the capacity of these dolphins to adapt to local environmental conditions combined with social facilitation of resource exploitation (Hoelzel *et al.* 1998; Natoli *et al.* 2005). Both the behavioural plasticity and the social structure of these dolphins support the idea that this hypothesis is plausible. Although a large repertoire of feeding behaviours have been observed for bottlenose dolphins (e.g. Shane *et al.* 1986), in specific habitats and areas individuals show specialised feeding strategies (e.g. Smolker *et al.* 1997; Barros and Wells 1998), which may be transmitted through vertical social learning (Krützen *et al.* 2005). In Port Stephens, strong-bonded related females and allied males appear to concentrate their activities, including foraging, in certain core areas of their range with specific habitat characteristics (Möller 2001; Möller *et al.* 2006). Although we do not have information on the feeding ecology of the dolphins we studied, differences in habitat characteristics between the embayment (extensive seagrass beds and mangroves) and along the open coast (predominantly sandy beaches and rocky reefs) suggest that differences in prey and feeding specialisations between embayment and coastal dolphins are likely to occur. Differences in prey specialisation and foraging habitats between inshore and nearshore common bottlenose dolphins have been well documented for the Gulf of Mexico (Barros and Wells 1998), and also suggested as a potential mechanism for the genetic differentiation found between dolphins of the two types of environments (Sellas *et al.* 2005).

Here, we document remarkably fine-scale, moderate genetic structure at microsatellite DNA within a few tens of kilometres. This structure, however, is unlikely to be an artefact of the embayment being inhabited by only one or a few extended family groups, as studies on the genetic relatedness of embayment dolphins showed that the social groups of males and females are formed, respectively, by randomly related individuals and individuals from different maternal lineages (Möller *et al.* 2001, 2006). In addition, the overall F_{IS} for the embayment population was negative and non-significant (data not shown), suggesting that the level of co-ancestry within the population is negligible. Although the recent availability of the embayment environment during the Holocene may have provided the opportunity for partial geographic isolation from open coastal waters, ecological and behavioural factors probably played a major role in promoting and maintaining genetic subdivision between dolphins of the two environments. These include high site fidelity to the local area (known from photo-identification studies, e.g. Möller *et al.* 2002), and the potential development of resource and behavioural specialisations in relation to prey and feeding (e.g. Hoelzel *et al.* 1998; Natoli *et al.* 2005; Sellas *et al.* 2005). Additional genetic studies on other highly mobile species distributed in both nearshore and inshore environments would be of great interest to verify whether the pattern of genetic differentiation observed here is common across our coasts or restricted to a few species and particular inshore environments.

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Appendix 1. Allelic frequencies for each bottlenose dolphin community at nine microsatellite loci and number of individuals genotyped at each locus

Alleles private to the coastal* and embayment** communities

Locus	*n*	Alleles									
EV1		*141*	*143*	*145*	*147*	*155*	*157**	*163*			
WPS	13	0.577	0.038	0	0.154	0.192	0	0.038			
EPS	20	0.625	0	0.025	0.225	0.1	0	0.025			
NC	19	0.237	0	0.079	0.632	0.026	0.026	0			
BI	17	0.235	0.059	0.235	0.382	0.059	0	0.029			
FOR	7	0.214	0	0.214	0.571	0	0	0			
KW2		*140*	*142*	*144*	*146*	*156*	*158*	*160*	*162*	*164**	
WPS	15	0.033	0.067	0.233	0.033	0.167	0	0.367	0.1	0	
EPS	20	0	0.05	0.15	0.05	0.175	0.025	0.475	0.075	0	
NC	19	0.079	0.237	0.184	0.053	0.237	0.026	0.079	0.105	0	
BI	21	0.024	0.286	0.167	0.071	0.167	0.048	0	0.167	0.071	
FOR	9	0	0.167	0.167	0	0.111	0	0	0.111	0.444	
KW12		*168*	*170*	*176*	*178*	*180*	*188**				
WPS	14	0	0.071	0.107	0	0.821	0				
EPS	20	0.05	0.25	0.25	0.025	0.425	0				
NC	19	0.132	0.263	0.316	0.026	0.237	0.026				
BI	20	0	0.2	0.45	0.05	0.3	0				
FOR	10	0	0.2	0.55	0	0.25	0				
MK6		*148*	*150**	*154*	*160*	*162***	*164**	*168**	*170**		
WPS	15	0.133	0	0.233	0.6	0.033	0	0	0		
EPS	19	0.105	0	0.158	0.737	0	0	0	0		
NC	20	0.15	0.05	0.1	0.625	0	0	0.025	0.05		
BI	20	0	0.2	0.15	0.6	0	0.025	0.025	0		
FOR	10	0.1	0.3	0	0.5	0	0	0.1	0		
D8		*96**	*100*	*104*	*106*	*108**	*112*	*114**			
WPS	15	0	0	0	0.033	0	0.967	0			
EPS	20	0	0.025	0.05	0.075	0	0.85	0			
NC	20	0	0.075	0.1	0.025	0	0.8	0			
BI	21	0	0.214	0.167	0.048	0	0.548	0.024			
FOR	10	0.05	0.2	0.05	0.15	0.05	0.45	0.05			
EV37		*202*	*204*	*206*	*208*	*218*	*224**	*228**	*230**	*234**	*236**
WPS	15	0.6	0	0.167	0.033	0.2	0	0	0	0	0
EPS	20	0.65	0.075	0.1	0.1	0.075	0	0	0	0	0
NC	20	0.625	0.1	0.075	0.05	0	0.075	0.025	0	0.025	0.025
BI	21	0.5	0.024	0.167	0.238	0.024	0	0.024	0.024	0	0
FOR	10	0.55	0.15	0.2	0.1	0	0	0	0	0	0
MK5		*214*	*216*	*218*	*224**	*226*					
WPS	13	0.115	0.846	0	0	0.038					
EPS	20	0.1	0.825	0.05	0	0.025					
NC	19	0.105	0.737	0	0	0.158					
BI	20	0.2	0.65	0.075	0.025	0.05					
FOR	10	0.2	0.7	0.1	0	0					
MK8		*96**	*100**	*104*	*106**	*108*	*112*	*114**	*116*		
WPS	14	0	0	0.5	0	0.464	0.036	0	0		
EPS	20	0	0	0.35	0	0.625	0	0	0.025		
NC	19	0.026	0.026	0.605	0.079	0.184	0.026	0	0.053		
BI	21	0.048	0	0.5	0.071	0.31	0	0.048	0.024		
FOR	10	0	0	0.55	0.1	0.2	0	0	0.15		
MK9		*178*	*180*	*182*	*184**						
WPS	14	0.036	0.143	0.821	0						
EPS	20	0	0.3	0.7	0						
NC	20	0.05	0.225	0.7	0.025						
BI	20	0.05	0.175	0.625	0.15						
FOR	8	0.062	0.438	0.5	0						