



Evidence of genetic isolation by habitat fragmentation in Indo-Pacific humpback dolphins (*Sousa chinensis*) from Central Queensland, Australia.



Daniele Cagnazzi and Peter Harrison,
Marine Ecology Research Centre, Southern Cross University
Lismore, Australia

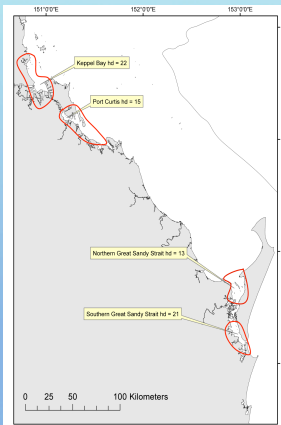
Abstract

In this study we provide evidence of genetic differentiation by habitat fragmentation in humpback dolphin, *Sousa chinensis*. 73 biopsy samples were collected from 4 neighbour populations in Queensland: Keppel Bay (KB), Port Curtis (PC), Northern Great Sandy Strait (NGSS) and Southern Great Sandy Strait (SGSS). KB-PC and NGSS-SGSS pairs are separated by about 30 km, while PC and NGSS are 300 km apart. Habitat fragmentation by sedimentation was identified as the main cause of the highly structured society recorded in the GSS. Data from 27 microsatellite loci were used to compare the degree of genetic differentiation among populations. High levels of genetic differentiation was found among populations ($F_{ST} = 0.08$; $R_{ST} = 0.19$). Pairwise comparison tests showed significant differentiation among NGSS and SGSS but not among KB-PC. Population assignment test confirmed those results. Analysis of genetic similarity vs. geographical distance was not significant ($r = 0.82$, $P = 0.08$), with gene flow among PC-NGSS higher than among NGSS-SGSS regardless of distance. Comparison of allelic richness showed that the genetic variability in SGSS was significantly lower than in other populations. We conclude that habitat fragmentation may have limited the dispersal capabilities of humpback dolphins between the NGSS-SGSS, thereby reducing gene flow among the two populations.

Methods

Data collection. Between 2007 and 2008, a total of 73 tissue samples of humpback dolphins were collected from 4 geographically separate neighbouring populations along the Queensland coast: Northern Great Sandy Strait (NGSS) and Southern Great Sandy Strait (SGSS) in Southern Queensland (Cagnazzi et al. 2009), and Port Curtis and Keppel Bay in Central Queensland (Fig 1).

Hypothesis testing. The NGSS-SGSS and PC-KB populations pairs are divided by two narrow channels each of about 30 km in length. Due to the increasing sedimentation the channel between the NGSS and SGSS populations is often obstructed, while the channel between KB and PC is crossable in almost all tide conditions. In this project the null hypothesis of no genetic differentiation among neighbor humpback dolphins populations was tested against the alternative hypothesis of extremely localised genetic differentiation due to habitat fragmentation.



Sampling technique

Samples were collected using the PAXARMS system (Krützen et al. 2002), a biopsy system developed to collect skin samples from small cetaceans. This system consists of a modified 0.22 calibre rifle with a larger barrel to fit the biopsy darts that are made out of polycarbonate with stainless steel biopsy tips. Biopsy heads have a diameter of 5 mm and a length of 8 mm to keep the amount of material collected to the minimum size suitable for genetic study, and to guarantee dolphin welfare.

DNA extraction

Total DNA was extracted from approximately 5-10 mg of tissue following the instruction provided with the DNA purification protocol of mouse tail tissues in the Genra Puregene Kit (QIAGEN).

Figure 1. Geographic distribution of the 73 biopsy samples of humpback dolphins collected between 2008 and 2009

Microsatellite genotyping

Samples were genotyped at 27 microsatellite loci, previously used in many cetacean population structure studies. Eight different dinucleotide microsatellite loci (Krützen et al. 2001, Valsecchi and Amos 1996, Hoelzel et al. 1998, Shinohara et al. 1997), and 19 tetranucleotide loci (Nater et al. 2009) were screened.

Microsatellite data analysis

Data quality control and genetic variability

Scoring error was evaluated by repeating amplification and scoring for 10% of samples at all loci. All samples were tested for possible duplicates in CERVUS (Marshall 1998). All microsatellite genotypes for the remaining samples were screened for the presence of irregular repeat unit length, large allele dropout and null alleles using MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004). Departure from Hardy-Weinberg equilibrium was tested in the program Arlequin 3.1 (Excoffier et al. 2005). Genotypic linkage disequilibrium was tested in GENEPOP. P-values were estimated using a Markov chain method (Raymond and Rousset 1995). Because of multiple comparisons, Bonferroni corrections (Rice 1989) were applied. The Allelic richness, observed heterozygosity (H_o), and expected heterozygosity (H_e), were calculated using FSTAT 2.9.3 (Goudet 2001).

Population structure and dispersal analysis

Genetic differentiation was investigated using both F_{ST} statistic, based on the infinite alleles model (Weir & Cockerham 1984) and R_{ST} statistic based on the step-wise mutation model (Slatkin 1995). The AMOVA was used to examine the partitioning of genetic variation within and among the geographic regions, Capricorn Coast (KB and GLD) and Great Sandy Strait (NGSS and SGSS) and within and among populations (KB, GLD, NGSS and SGSS). The programme Arlequin was used to run the AMOVA. Differentiation among pairs of populations was further investigated with the population comparison module. Gene flow between pairs of localities (N_m) was estimated from F_{ST} , R_{ST} using the private allele method as described in Slatkin (1995). Population structure was further investigated using a Bayesian algorithm implemented in STRUCTURE (Pritchard et al. 2000). Population structure was investigated using "admixture model". This model was run without a priori information on sampling locality. To identify the best number of putative populations (K), that explains the observed genetic differentiation, the admixture model was first run with only 100,000 iterations and initial burn-in of 10,000. The number K was increased until posterior probability, $\ln Pr(X|K)$, started decreasing constantly. The final structure was assessed running the model for 1,000,000 iterations with an initial burn-in of 100,000 for K values varying from 1 to the value that maximised the posterior probability. Five independent tests were run to test the consistency of the results.

Results

Source of variation	d.f.	SSD	var	% var	F	P
<i>Distance method: number of different alleles (F_{ST})</i>						
Among regions (F_{ca})	1	31.53	0.30	8.07	0.08	Hs
Among populations within region (F_{sc})	2	21.41	0.24	6.32	0.06	Hs
Within populations (F_{st})	126	412.20	3.27	85.60	0.14	Hs
Total	129	465.15	3.82			
<i>Distance method: sum of squared size difference (R_{ST})</i>						
Among regions (F_{ca})	1	256.27	3.03	18.35	0.18	Hs
Among populations within region (F_{sc})	2	109.57	1.38	8.39	0.10	Hs
Within populations (F_{st})	126	1526.77	12.11	73.27	0.26	Hs
Total	129	1892.6	16.53			

Table 1 Variance components and permutation probabilities for AMOVAs. Dataset was partitioned according to regions (Capricorn Coast and Great Sandy Strait). df = degrees of freedom, SSD = sum of squares, permutation probability is given for the probability that randomised value > observed value. Results for both evolution models are presented.



Figure 2. Picture taken during a successful attempt showing the dart in red near the top of the picture, just before it hits the dolphin.

Source of variation	df	SSD	Variance	% variation	P
Among populations F_{ST}	3	52.95	0.45	12.11	0
Within populations	126	412.20	3.27	87.89	
Total	129	465.15	3.72		
Among populations R_{ST}	3	2189.07	19.94	17.59	0
Within population	126	11773.5	93.44	82.41	
Total	129	13962.6	113.38		

Table 2. AMOVAs when dataset was partitioned according to populations (KB, GLD, NGSS, SGSS). Results are present for both evolution models.

Comparisons	Keppel Bay	Port Curtis	NGSS	SGSS
Keppel Bay	\	0.031 (7.5)	0.180 ** (1.13)	0.275** (0.65)
Gladstone	0.048** (4.87)	\	0.110 * (2.01)	0.252** (0.73)
NGSS	0.129** (1.67)	0.104** (2.13)	\	0.115** (1.91)
SGSS	0.159** (1.32)	0.166** (1.24)	0.094** (2.4)	\

Table 3. Differentiation among pairwise populations using F_{ST} values (lower diagonal) and R_{ST} (upper diagonal) (**P < 0.05, ***P < 0.0001). N_m values shown in parentheses.

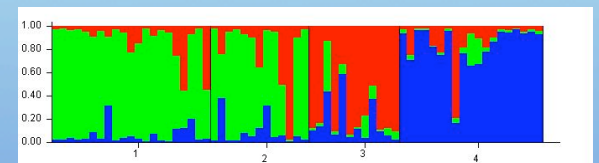


Figure 2 Structure plots showing estimated proportions of the coefficient of admixture of each individual's genome that originated from $K = 3$ (best model). Geographical origin of the samples is given below the graphic. The numbers 1-4 indicate the sampling location being KB, PC, NGSS and SGSS respectively.

SPECIAL THANKS

This project has been conducted under a scientific research permits granted from GBRMPA, QPWS and DEWHA. Special thanks go to Michael Krützen, Anthropology Institute and Museum, Zurich University for his expertise and for the access to labs and facilities. Last thanks go to all the volunteers that participated in this project.