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Population Genetic Structure of Giant Clams, *Tridacna gigas* (Family Tridacnidae), on the Great Barrier Reef

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Abstract: The Great Barrier Reef (GBR) is one of the few remaining Pacific reef systems where the giant clam, *Tridacna gigas*, can be found in large numbers. Genetic data from Inter-Simple Sequence Repeat loci (ISSR) suggest low levels of genetic structuring among four north-central GBR populations (Phi_{PT} = 0.0322, p = 0.005), with significant genetic differences evident between populations from Thetford and Grub Reefs. The data suggests a weak correlation of genetic differentiation among populations and their linear distance of geographical separation. The region of the GBR sampled here is approximately a quarter of the total distribution of *T. gigas*. Significant genetic structuring may therefore be present between northern and southern GBR *T. gigas* populations and consequently, a conservative approach should be practiced relating to the translocation of this species throughout the GBR.

Key words: DNA, bivalve mollusc, ISSR, polymorphic, gene flow

Introduction

The giant clam, *Tridacna gigas,* is the world's largest bivalve and one of nine species of tridacnid clam found in waters of the Great Barrier Reef. Historically, *T. gigas* occurred on coral reefs throughout the Indo-Malay region eastwards to Micronesia in the central Pacific (Yonge, 1975). However, recent habitat destruction and over-exploitation by Pacific islanders who use this species as a traditional food source, has led to localized extinction over much of its former range (Copland and Lucas, 1988). As a result of the global decline in *T. gigas* populations the species is classified by the IUCN as vulnerable (VU-A2cd) and is listed in Appendix II of CITES in order to control the trade and export of the species.

The Great Barrier Reef (GBR), Queensland, Australia, is the world's largest marine reserve and is one of the few remaining regions where large populations of *T. gigas* are still found. The presence of giant clams on the GBR acts as an attraction to large numbers of tourists and consequently has led in recent years to requests by several tourism operators for the movement of clams to reefs where they are not as abundant. In addition, in the mid 1980's there was a significant push to establish an aquaculture industry based around this species on the GBR (Crawford *et al*., 1986; Lucas, 1994). There has been resistance from the regulatory body responsible for sustainable management of the GBR to these requests, however, with their major concern being that translocation of giant clams throughout the GBR might impact on local genetic diversity.

To date there has only been one documented study that has examined the genetic architecture among giant clam populations from different patches of reef on the GBR (Benzie and Williams, 1992). This study, based on allozyme variation, found little evidence for genetic differentiation between reef populations separated by more than 1000 km. However, as demonstrated by a large number of

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population genetic studies involving marine organisms, failure to detect significant population differentiation using allozymes does not necessarily signify that genetic structuring is absent, as allozymes often exhibit low levels of inter-population polymorphism due to balancing selection (Avise, 1994, Ward and Grewe, 1994; Hilbish, 1996; Shaklee and Bentzen, 1998). Karl and Avise (1992) cite numerous examples where data from allozyme loci for marine species suggested little or no population subdivision, only for mtDNA studies to later reveal discontinuities (Ovenden and White, 1990).

Given the importance of understanding genetic processes and levels of population connectivity to the formulation of a sound translocation management plan for this species, we reanalyzed those samples collected in Benzie and Williams (1992) survey using a more powerful DNA-based approach. In the present study we examined genetic variability at four Inter-Simple Sequence Repeat loci to test the hypothesis that giant clams on the GBR represent a single panmictic genetic unit. This is the first exploration into the population structuring of this species using molecular DNA data.

Materials and Methods

Sample Collection

Between 27 and 40 individual clams were sampled from each of two sites on each of six reefs in the GBR throughout 1990 and stored at -80°C until analysis (Benzie and Williams, 1992). Of these samples, between 14 and 22 individuals from each of four reefs were made available for the present study (Fig. 1).

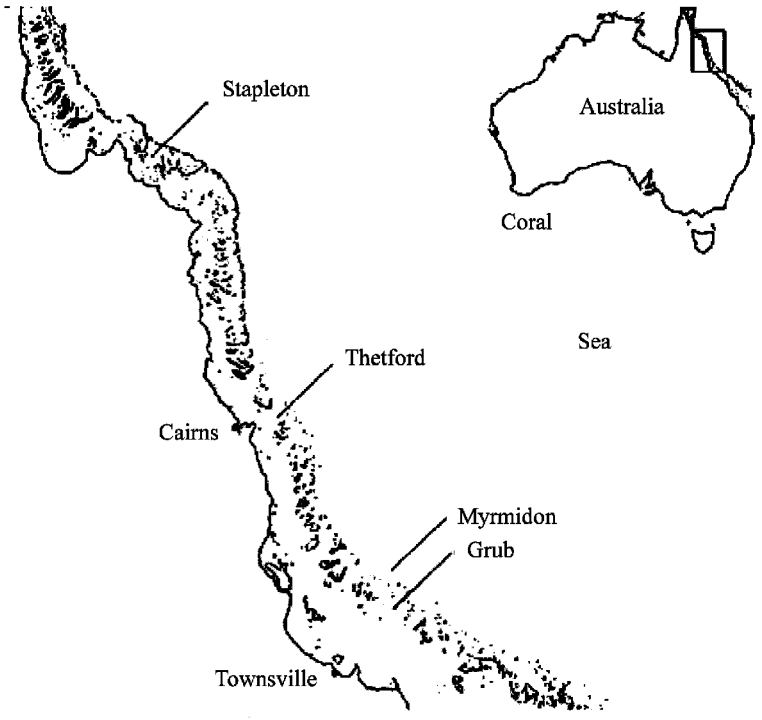

Fig. 1: Giant clam *Tridacna gigas* populations from the Great Barrier Reef surveyed by ISSR loci in the present study

Table 1: Inter-Simple Sequence Repeat (ISSR) primers tested for amplification of the *Tridacna gigas* genome. *signifies the primers utilized for this study. Y = C, T; R = A, G; V = A, C, G

Primer name	Primer sequence	GC content (%)	Temp (°C)
ISSR807	(AG)8-T	47	49.9
ISSR808*	(AG)8-C	53	52.3
ISSR809	(AG)8-G	53	52.3
ISSR810*	(GA)8-T	47	49.9
ISSR811*	(GA)8-C	53	52.3
ISSR812*	(GA)8-A	47	49.9
ISSR842	(GA)8-YG	55/50	51.3
ISSR817	(CA)8-A	47	49.9
ISSR818	(CA)8-G	53	52.3
ISSR816	(CA)8-T	47	49.9
ISSR847	(CA)8-RC	55/50	51.3
ISSR848	(CA)8-RG	55/50	51.3
ISSR825	(AC)8-T	47	49.9
ISSR826	(AC)8-C	53	52.3
ISSR827	(AC)8-G	53	52.3
ISSR855	(AC)8-YT	50/44	51.6
ISSR856	(AC)8-YA	50/44	51.6
ISSR857	(AC)8-YG	55/50	51.3
ISSR843	(CT)8-RA	50/44	51.6
ISSR844	(CT)8-RC	55/50	51.3
ISSR845	(CT)8-RG	55/50	51.3
ISSR886	VDV(CT)7	~50	50.4
ISSR801	(AT)8-T	0	32.2
ISSR802	(AT)8-G	6	33.0
ISSR803	(AT)8-C	6	33.0
ISSR831	(AT)8-YA	0/6	32.6
ISSR832	(AT)8-YC	6/11	35.6
ISSR833	(AT)8-YG	6/11	35.6
ISSR804	(TA)8-A	0	32.2
ISSR805	(TA)8-C	6	33.0
ISSR806	(TA)8-G	6	33.0
ISSR837	(TA)8-RT	6	33.0
ISSR838	(TA)8-RC	6/11	35.6
ISSR839	(TA)8-RG	6/11	35.6

Laboratory Analysis

The giant clam samples of Benzie and Williams (1992) were reanalyzed using Inter-Simple Sequence Repeat (ISSR) loci. ISSR analysis relies on PCR amplification using microsatellite sequences as primers to generate multi-locus markers. As highlighted by Reddy *et al.* (2002), ISSR's combine the advantages of microsatellites with the ease of Amplified Fragment Length Polymorphism (AFLP) analyses. DNA was extracted from all tissues using the modified CTAB protocol of Evans *et al.* (2004) and tested for ISSR amplification using 34 primer pairs (Table 1). All but one of the primer pairs were anchored at the 3' end, the exception being primer ISSR886, which was 5' anchored by three degenerate bases. All primers comprised eight di-nucleotide repeats with 18 anchored by a single selective base and 15 by a pair of selective bases. In many cases one of the selective bases was an R or Y representing purines and pyrimidines respectively. A single primer pair was used in each PCR, which was performed in a total volume of 25 μL. Reactions contained 2.5 μL of 10× PCR buffer (Qiagen), 1 μL of $MgCl_2$ (25 mM), 0.5 μL of dNTPs (10 mM each), 0.8 μL of primer (10mM), 0.1 μL Taq DNA polymerase (10 u μL^{-1}), 1 μL of DNA template (~20 ng) and 19.1 μL of water.

PCR amplifications were performed in 48 well plates on an Eppendorf mastercycler using the following thermal conditions: a hot start of 94°C for 3 min; followed by 35 cycles of denaturation at 94°C for 1 min; annealing at 50°C for 1 min; extension at 72°C for 2 min and product extension at 72°C for 5 min.

Five microliters of each ISSR amplification product was initially assessed by electrophoresis on 2.5% agarose gels containing 1×TBE solution and 3 μL of ethidium bromide per 100 mL (in buffer and gel). All gels were run at 100 V for 45 min with a 1 Kb size standard ladder (Promega).

Four primers (ISSR808, ISSR810, ISSR811, ISSR812) produced consistently scoreable alleles and were subsequently synthesized with an incorporated 5' fluorescent label. ISSR amplification products were mixed with a loading buffer, denatured at 94°C for 2 min and run for 90 min on 4% denaturing polyacrylamide gels containing 30 μL Temed and 75 μL of 10% Ammonium per-sulfate solution. Alleles were visualized and sized using a GELSCAN (Corbett Research) genotyper and GENESCAN ROX2500 size standard.

Data Analysis

Polymorphic bands were scored qualitatively for their presence (1) or absence (0) in each sample. All supplementary statistical analyses were performed using the software package GenALEx (Peakall and Smouse, 2001). Using this package, pair-wise binary genetic distances were estimated for dominant data following the method of Huff *et al*. (1993). This method is particularly appropriate for binary genetic data as it provides an Euclidian distance metric and is therefore suitable for ensuing Analysis of Molecular Variance Analyses (AMOVA) (Excoffier *et al*., 1992; Huff *et al*., 1993; Peakall *et al*., 1995). To account for the increased probability that a single test within a multiple test analysis will be significant purely due to chance, all multiple tests were adjusted for significance ($\alpha<0.05$) by the sequential Bonferroni method (Rice, 1989).

Given the relatively linear spatial sampling regime of giant clam populations in the present study conformance of the data to an isolation by distance model was examined using a Mantel test (Smouse and Long, 1992). A principal coordinates analysis was also performed to plot the genetic relationship between individuals from the various sampled reef systems.

Results

Giant clam populations demonstrated moderate levels of polymorphism at the four ISSR loci, with 21 alleles present in the Stapleton, Thetford and Mymridon samples. However, in the Grub Reef population only 20 alleles were found, probably purely as a result of sampling error. Alleles were ubiquitously distributed among the sample sites, with no clam population possessing unique alleles. Moderate levels of genetic variability were present within populations, with mean heterozygosity estimates ranging between 0.28 and 0.37 (Table 2).

The ISSR data suggested a latitudinal effect on genetic relatedness among the various reef populations, with populations in close geographical proximity also the most genetically similar. For example, the Myrmidon and Grub Reef populations were the most geographically proximate of those sampled in the study, with a corresponding genetic distance of 0.024. In contrast, those populations geographically distant from Grub Reef exhibited higher estimates of genetic dissimilarity, with the greatest disparity exhibited between clams sampled from Thetford and Grub Reefs (0.062) (Table 3).

Table 2: Number of polymorphic ISSR bands, number of bands restricted (unique) to only a single population and mean heterozygosity (±SE) for four giant clam populations sampled from the Great Barrier Reef. n = number of giant clam individuals sampled from each reef population

Reef Population	Stapleton (n = 14)	Thetford (n = 20)	Myrmidon (n = 22)	Grub (n = 22)
Polymorphic bands	21	21	21	20
Private bands	0	0	0	0
Mean heterozygosity	0.28 (±0.04)	0.32 (±0.04)	0.37 (±0.03)	0.34 (±0.04)

Table 3: Pair-wise genetic distances (Huff *et al*., 1993) derived from four ISSR loci among *Tridacna gigas* populations from the Great Barrier Reef

	Stapleton Rf	Thetford Rf	Myrmidon Rf	Grub Rf
Stapleton Rf	-			
Thetford Rf	0.042	-		
Myrmidon Rf	0.052	0.032	-	
Grub Rf	0.058	0.062	0.024	-

Table 4: Pair-wise population Phi_{PT} values for four *Tridacna gigas* populations from the Great Barrier Reef. Phi_{PT} values are below diagonal, whilst probability values based on 999 permutations are shown above diagonal. [a]Only the pair-wise comparison between Thetford and Grub Reefs were deemed significant after sequential Bonferroni adjustment (Rice, 1989)

	Stapleton Rf	Thetford Rf	Myrmidon Rf	Grub Rf
Stapleton Rf	-	0.149	0.047*	0.014*
Thetford Rf	0.024	-	0.235	0.001***[a]
Myrmidon Rf	0.040	0.012	-	0.470
Grub Rf	0.058	0.069	0.000	-

*significant at $p<0.05$, *** $p<0.001$ before Bonferroni adjustment

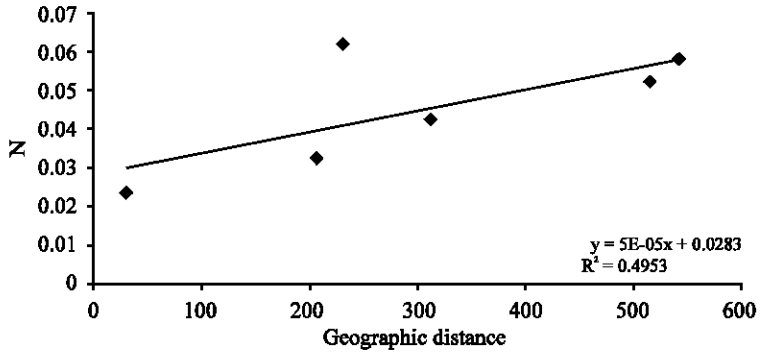

Fig. 2: Relationship between geographic distance (measured as a straight line distance) and genetic distance (Huff *et al*., 1993) for four Great Barrier Reef populations of *Tridacna gigas*

A trend towards increased genetic distance vs geographical distance was suggested by the data (Fig. 2), although the Mantel's test found no significant correlation between these two variables ($p>0.05$). This regression, however, is likely to be strongly influenced by the large genetic distance between Thetford and Grub Reefs, despite these two populations not being the most geographically disparate of those sampled.

Analysis of Molecular Variance suggested that there is small scale genetic structuring among GBR giant clam populations, with around 3% of the total genetic variation in the data set attributable to differences among clam populations. Although the estimate of Phi_{PT} was relatively small, it was nonetheless significant ($Phi_{PT} = 0.0322$, $p = 0.005$). As with genetic distance, pair-wise estimates of population structuring (Phi_{PT}) demonstrated that most of the genetic variability among reef populations appeared to be related to spatial structure, with larger Phi_{PT} values associated with greater geographical distance among reefs. Significant Phi_{PT} estimates were observed for comparisons among Mymridon and Stapleton, Grub and Stapleton and Grub and Thetford Reef populations. However after Bonferroni adjustment only the comparison between Grub and Thetford Reef was still deemed significant (Table 4).

Principal coordinates analysis (Fig. 3) revealed no distinct grouping of giant clams from individual reef systems, or an obvious association between geographically neighboring populations.

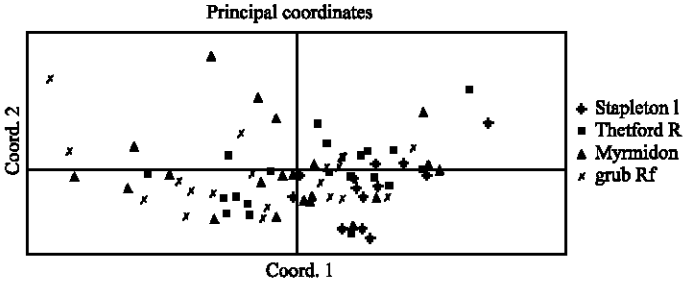

Fig. 3: Principle coordinates analysis based on genetic distance (Huff *et al*., 1993) between *T. gigas* individuals sampled from four reef populations on the Great Barrier Reef

Discussion

The present study found high levels of variability within populations of *Tridacna gigas* from the GBR, which is in agreement with earlier allozyme data (Benzie and Williams, 1992). However, in contrast to the earlier study our ISSR data seems to suggest low levels of genetic structuring within the GBR, particularly in relation to spatial positioning of populations. This conclusion is based on a small, but nonetheless significant population-wide estimate of Phi_{PT}.

Although small scale structuring was suggested by the data set, unfortunately we can not conclusively reject the null hypothesis of there being no genetic structuring among giant clam populations on the GBR. This was mainly due to two reasons. Firstly, although small levels of genetic structuring were evident in three of the six Phi_{PT} population comparisons, after Bonferroni correction only that of Thetford and Grub Reefs were still deemed significant. While the Bonferroni correction (Rice, 1989) is a conservative adjustment (Ryman and Jorde, 2001) the fact that two of the three populations ended up not being significant probably signifies how little true genetic differences exist among geographically proximate clam populations. Secondly, sample sizes were lower than we desired due to poor preservation. For many individuals genomic DNA was severely degraded and we couldn't amplify the ISSR loci of interest. Lower than desired sample numbers obviously reduced the statistical power of the loci to detect larger levels of population differentiation. In our opinion, studies based on larger sample numbers and using more powerful molecular techniques such as microsatellites or mtDNA may be needed to fully resolve the question of just how structured giant clam populations are.

One trend that our ISSR data set does suggest, however, is that across the length of the GBR there may be important genetic differences among clam populations related to geographical separation. Despite the low power of our data set to statistically detect isolation by distance using a Mantels test, there was a conspicuous trend towards increased genetic distance when spatial distance among reef patches also increased. One important factor to consider is that the reef populations sampled in our study were separated from each other by only a maximum of 550 km. This represents only around a quarter of the total distributional range of *T. gigas* on the GBR. Given that the spatial distance among sampled populations represents such a small area of the total species range, it is highly likely that if this trend is magnified across the entire GBR *T. gigas* populations denizen to the northern and southern extremities will be significantly genetically differentiated, purely as a function of geographical distance effects. Support for this hypothesis is that genetic differentiation over large spatial scales has been observed in *T. gigas* in French Polynesia (Laurent *et al*., 2002), as well as in the closely related species *T. derasa* on the GBR. This later species has a similar distribution and reproductive biology as *T. gigas* and on the GBR geographically proximate populations of *T. derasa* were found to exchange

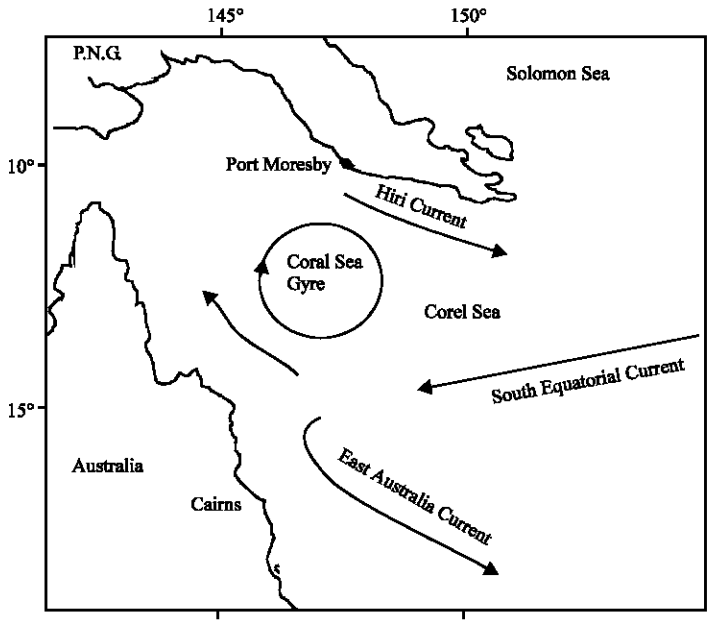

Fig. 4: Bifurcation of the South Equatorial Current between 14-18°S as it hits the Great Barrier Reef. The South Equatorial Current bifurcates approximately equally into the southerly flowing East Australian Current and a northerly flowing current that flows along the northern Queensland coastline into the Gulf of Papua

high levels of gene flow. Significant differentiation as a consequence of rare allele frequency differences, however, was observed when reef populations were pooled into northern and southern GBR regions (Macaranas *et al*., 1992).

T. gigas has a passive larval duration of approximately 10 days and reproduces during the Austral summer (Copland and Lucas, 1988), when net southerly currents are prevalent over much of the north-central GBR (i.e., the region encompassing our sampling sites) (Williams *et al*., 1984). It was these conditions that were used as supporting evidence of panmixia in giant clam populations on the GBR by Benzie and Williams (1992). The northern most sampling site in this study of Stapleton Reef, however, is situated close to the divergence point in the westward flowing South Equatorial Current as it hits the GBR (at between 14 and 18°S) (Fig. 4). At the point on the GBR approximately represented by the Stapleton sample, the South Equatorial Current bifurcates; with roughly equal volumes flowing south along the Queensland coast into the East Australian Current and north along the far Northern Queensland coastline into the Gulf of Papua (Fig. 4) (Andrews and Clegg, 1989; Dennis *et al*., 2001). This bifurcation point may therefore represent a significant determinate of the subsequent direction of gene flow among populations, with transmission of genes predominantly moving in a northerly direction above ~18°S and in a southerly direction below ~14°S. If the flow of gene flow is indeed partitioned depending on the position of reef patches in relation to the South Equatorial Current we could expect that significant genetic population structure would be found among populations at the north-south extremities of the GBR. Wider sampling of reef populations on the GBR will be necessary to address this possibility.

The prospect of genetic structuring among spatially distant *T. gigas* populations has important implications for any future translocation management policies for this species. Rather than a single

large breeding population of *T. gigas* covering the length of the GBR as originally suggested by Benzie and Williams (1992), it appears possible that populations may be reproductively isolated with respect to distance and therefore may have developed local adaptation to their specific reef environment at the extremities of the distribution. While minimal genetic impact may result from moving clams between proximate reef patches, genetic pollution and possible disruption of adaptive genome complexes (Carvalho, 1993) may result if clams are translocated more than a few hundred kilometers from their natal reef patch. Therefore in the absence of more conclusive data, a conservative management strategy where giant clams are not moved further than the neighboring reef patch is to be recommended to prevent possible adverse genetic effects on locally adapted populations.

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