GENETIC STRUCTURE OF GIANT CLAM, *TRIDACNA MAXIMA* IN THE ANDAMAN SEA, THAILAND

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ABSTRACT

Three populations of giant clam, *Tridacna maxima*, from Surin, Phuket and Adang Rawii islands were examined for allozyme variation by starch-gel electrophoresis. Four polymorphic loci showed high heterozygosity with the mean values of 36 %, 53 %, and 52 % for Surin, Phuket, and Adang Rawii respectively. The Nei's genetic distance over all loci showed that population difference between Surin-Adang Rawii was highest (0.09132) whereas lesser difference was found between Phuket-Adang Rawii (0.06346) and Surin-Phuket (0.04836). The genetic variation among the populations (Fst) showed no divergency between Surin-Phuket whereas significant differences were found between Phuket-Adang Rawii and Surin-Adang Rawii when the genotypic distribution between populations were tested. The results reveal that *T. maxima* in the area is divided into two sub-populations which may be explained by the specific water circulation pattern in the Andaman Sea. Two opposite currents meet and deflect in the vicinity of Phuket Island and this could prevent dispersal of larvae between the north and the south water body. The consequences of restocking or introducing giant clams from other sources should be considered in order to preserve the genetic identity of local sub-populations.

INTRODUCTION

Four species of giant clams; *Tridacna gigas*, *T. squamosa*, *T. maxima*, and *T. crocea* have been reported from the west coast of Thailand. Due to the appreciation of giant clams as food and the value of their shell, giant clams have become threatened organisms; *T. gigas* has not been reported for several years and is probably extinct, live specimens of *T. squamosa* are rarely found, only *T. maxima* and *T. crocea* are locally abundant (S. Chantrapornsyl, pers. comm.). Even though collecting of giant clams has been illegal in Thailand since 1992, the number of giant clams has still not recovered, probably due to irregular recruitment (Pearson & Munro 1991; Braley 1988).

Since exploitation of giant clams in natural populations proceed and the attempt to mass culture giant clams at Prachuab-Khiri-Khan Brackish water fisheries station Thailand has been successful (Nugranad, *et al.* 1996), restocking is considered. However, information on genetic structure of natural populations is needed for planning of restocking. In this study I investigated the genetic structure of three populations of *T. maxima* along the west coast of Thailand.

MATERIALS AND METHODS

Sampling

The mantle tissue from 42-116 individuals were sampled from three locations (Fig. 1) employing biopsy technique described by Benzie & Williams (1992a). SCUBA divers cut a small piece of mantle approximately one square centimetre and placed it in a plastic bag with sea water where it was kept during the dive. The samples were drained from sea water and frozen in liquid nitrogen or on dry ice during the collecting trips. The details on species, size and colour of both mantle and inner shell lips were noted onto the plastic bag.

Electrophoresis

Twenty enzymes were tested for electrophoretic polymorphism on horizontal starch gels by the techniques of Selander *et al.* (1971) and Richardson *et al.* (1986). The mantle tissue was homogenised in an equal

volume of 0.04 % mercaptoethanol, and centrifuged at 15,000 rpm for 15 minutes at 4 °C. The supernatant was soaked on to 9 x 4 mm paper wicks (Whatman 3 mm chromatography paper) and inserted in a 10.6 % starch gel. All enzymes were tested on two buffer systems (Tab. 1).

I. Electrode buffer: 0.30 M Sodium borate, pH 8.2
Gel buffer: 0.076 M Tris, 0.005 M Citric acid, pH 8.7
Running conditions: 250 V for four hours.

II. Electrode buffer: 0.223 M Tris, 0.086 M Citric acid, pH 6.3
Gel buffer: 0.008 M Tris, 0.003 M Citric acid, pH 6.7
Running conditions: 170 V for three hours.

Twelve of the enzymes showed activity, eight showed distinct bands, and four were selected for further analysis (PGI, MPI, DIA, and PGM) since they were polymorphic and consistently readable. Bands at a locus were designated numerically with the fastest band denoted "1", the second "2" and so on. In order to distinguish between the enzyme of the clam and the enzyme of the dinoflagellate symbiont (*Symbiodinium microadriaticum*), bands originating from the symbiont were identified by comparing the zymogram from symbiont free tissue (adductor muscle) to the zymograms from mantle tissue.

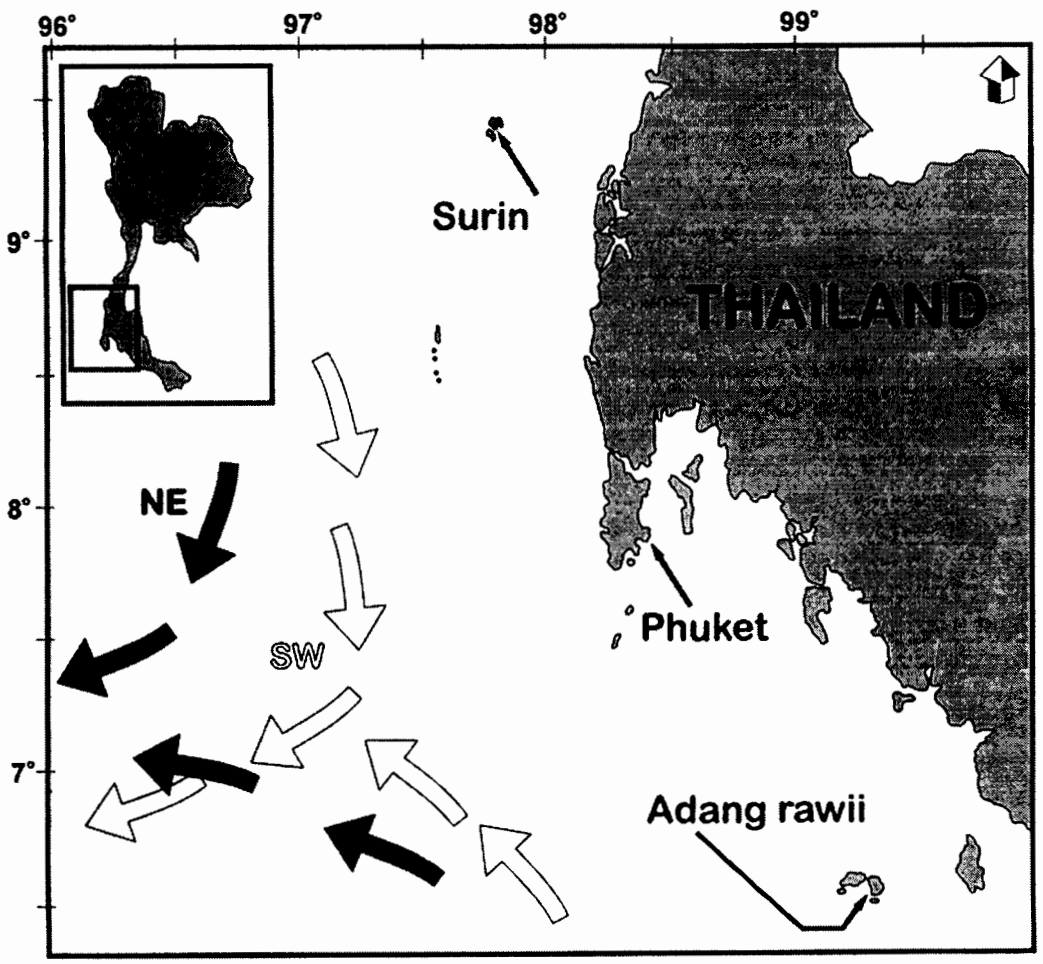

Figure 1. Current patterns off Phuket during the NE and SW monsoons. Study areas indicated.

Table 1. Results of preliminary screening for enzyme activity and polymorphism in *T. maxima*. The enzyme-reaction was tested on two buffer systems and selected buffers are given in parentheses. (-, No activity; +, Activity but poor resolution; ++, Readable; +++, Good resolution).

Enzyme-specific stains		E.C. number	Tris citrate	Poulik
Alcohol dehydrogenase	ADH	1.1.1.1	-	-
Alkaline phosphatase	ALKP	3.1.3.1	-	-
Adenylate kinase	AK	2.7.4.3	-	-
Diaphorase	DIA	1.8.1.4	++	(+++)
Esterase	EST	3.1.1.1	(++)	+
Fumarase	FUM	4.2.1.2	+	+
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	+	+
Aspartate aminotransferase	AAT	2.6.1.1	-	-
Glycerol-3-phosphate dehydrogenase	GPD	1.1.1.8	-	-
Isocitrate dehydrogenase	IDH	1.1.1.42	+	+
Malate dehydrogenase	MDH	1.1.1.37	(+++)	++
Malic enzyme	ME	1.1.1.40	-	-
Manose-6-phosphate isomerase	MPI	5.3.1.8	(+++)	++
Phosphoglucomutase	PGM	5.4.2.2	++	(+++)
Glucosephosphate isomerase	GPI	5.3.1.9	++	(+++)
6-Phosphogluconate dehydrogenase	6PGD	1.1.1.44	-	-
Superoxide dismutase	TO	1.15.1.1	++	(+++)
Xanthine dehydrogenase	XDH	1.2.1.37	-	-
L ibitol dehydrogenase	SORDH	1.1.1.14	+	+
L-lactate dehydrogenase	LDH	1.1.1.27	(++)	+

Data analysis

The computer program GENEPOP Version 2 (Raymond & Rousset 1995) was used for Hardy-Weinberg exact tests and the Fisher's exact tests for population differentiation. Estimation of the gene flow (N_em) was calculated using the DIST program distributed with GENEPOP. F-statistics were calculated with FSTAT computer program (Goudet 1996). The other genetic values were calculated according to below equations (Nei 1972)

Heterozygosity: where p_i is the frequency of the i^{th} allele in the locus.

The identity index: where X_i and Y_i are the frequency of the i^{th} allele of a given locus in the two populations

The identity index for overall loci =

$$I = \frac{\sum x_i \sum y_i}{\sqrt{\sum x_i^2 \sum y_i^2}}$$

The genetic distance D =

$$\frac{\sum\left(\sum x_i^2 \sum y_i^2\right)}{\sqrt{\sum\left(\sum x_i^2\right)\sum\left(\sum y_i^2\right)}}$$

RESULTS

Hardy-Weinberg exact test showed that there was an overall heterozygote deficiency. Heterozygosities were high at all loci in all populations (Tab. 2) with a mean observed and expected heterozygosity of 47 % and 65 % respectively. Observed heterozygosity was lower than expected at all loci in all populations.

Nei's genetic distance over all loci among the populations were fairly high especially between Surin-Adang Rawii which had the longest geographic distance (Tab. 3). When tested with the Fisher's exact test for population differentiation, the *F*st values expressed significant differences between populations from Phuket-Adang Rawii and Surin-Adang Rawii while there was no significant difference between Surin-Phuket. The estimated gene flow (N_em) between Surin-Phuket was 18.04 which is considered effective panmixis (more than 10, Slatkin 1993) while the gene flow between Phuket-Adang Rawii and Surin-Adang Rawii were less than 10 (Tab. 3).

DISCUSSION

High heterozygosity in giant clams has been

Table 2. Allele frequency and heterozygosity for the polymorphic loci of *T. maxima* from Surin, Phuket and Adang rawii. "n" is the number of individuals analyzed at each locus and each sites. "S" is Surin, "P" is Phuket and "A" is Adang rawii.

		Allele frequency								n	Heterozygosity (%)	
		1	2	3	4	5	6	7	8		Observed	Expected
PGI	S	0.024	0.155	0.107	0.071	0.214	0.107	0.262	0.060	42	67	84
	P	0.022	0.087	0.141	0.054	0.261	0.076	0.293	0.065	46	74	81
	A	0.05	0.1	0.05	0.225	0.25	0.025	0.25	0.05	20	85	83
MPI	S	0.303	0.539	0.158	-					38	21	60
	P	0.239	0.375	0.205	0.182					44	75	74
	A	0.268	0.442	0.203	0.087					69	67	69
DIA	S	0.122	0.561	0.244	0.073					41	37	61
	P	0.109	0.457	0.337	0.098					46	43	66
	A	0.043	0.056	0.284	0.112					116	45	59
PGM	S	0.045	0.545	0.409	-					11	18	56
	P	0.143	0.554	0.25	0.054					28	18	62
	A	0.05	0.869	0.081	-					80	13	24

Table 3. Summary data on genetic values between populations of T. maxima along the west coast of Thailand. "ns" :non significant, "*" :p<0.05, "**" :p<0.01

	Geographic distance (Km)	Nei's genetic distance	Fst	Gene flow (Cockerham & Weir 1993)
Surin-Phuket	195	0.04836	0.0010ns	18.04
Phuket-Adang rawii	170	0.06346	0.0286**	6.95
Surin-Adang rawii	355	0.09132	0.0393*	4.91

reported by many authors (Benzie & Williams 1992 a, b; Macaranas *et al.* 1992). However the mean observed heterozygosity in this study (47 %) is higher than what was reported by Benzie & Williams (1992 a) (36 %) and by Campbell *et al.* (1975) (20.2 %) in studies of *T. maxima* from the Great Barrier Reef. The difference in degrees of heterozygosity could be an effect of the different loci analysed in the studies or it could be because organisms living in tropical areas tend to have higher genetic variability than organisms from tropical-temperate areas. Ayala *et al.* (1975) studied 13 species of benthic invertebrates and found that those living in low latitudes have higher genetic variability (heterozygosity) than those living in intermediate to high latitudes.

The heterozygote deficiency in the analysed populations could be caused by several factors, *i.e.*, inbreeding, selective migration, and selection against heterozygotes. Since giant clam is a protandrous hermaphrodite (Lucas 1988; Braley 1988) there is a risk of self fertilisation. Furthermore, observation from reared broodstock of *T. squamosa* reveal that only 5 % of clams release eggs and 15 % release sperms in a single spawning (Jintana Nugranad, pers. comm.) which increases the risk of inbreeding. Regarding selective migration, the homozygous larvae may have a low dispersal capacity and therefore will remain in fairly high number in the spawning area. Heterozygous larvae, in contrast, may be more active and migrate to other areas which will result in heterozygote deficiency. The deficiency of heterozygote may also be caused by settling of larvae from nearby localities. Selection against heterozygotes could hypothetically be an alternative possibility, but since heterozygous advantage is common in molluscs (Mitton 1993) I consider this possibility less likely.

Nei's genetic distance between populations were in concordance with the geographical distances (Tab. 3). The values are relatively high (Fig. 2) compared to the genetic distance between populations of *T. maxima* from the Great Barrier Reef (Benzie & Williams 1992 a). These differences could reflect a disparity in larval dispersal caused

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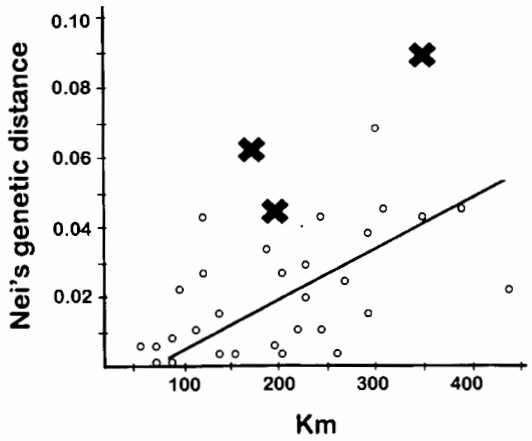

Figure 2. Nei's genetic distance as a function of geographic distance. Crosses show distances referred to in Tab. 3. Circles show data from Benzie & Williams (1992 a).

by the specific ocean currents, but it could also be an effect of differences in numbers and types of loci analysed in the two studies.

The genetic structure of *T. maxima* along the west coast of Thailand may be explained by the water circulation in this area. The study of Khokiattwong & Limpsaichol (1995 a, b) showed that a northern water mass circulates clockwise around the Nicobar Island while a southern water mass circulates counter clockwise around north of Sumatra. The two water masses encounter a mixing zone located west to southwest of Phuket during the northeast monsoon and proceed to southwest to south of Phuket during the southwest monsoon (Fig. 1). This divergent zone may prevent the dispersal of giant clam's larvae between the two water bodies.

Regarding restocking of *T. maxima* populations in the investigated area, the result indicates that transfer of individuals between Surin and Phuket would have none or minor effect on the genetic structure. However, the population at Adang Rawii differs significantly from the other two and should be restocked with individuals cultured from a native broodstock, or with individuals from a population of a closer genetic resemblance.

In summary, the result of this study indicate that restriction of gene flow between populations of *T. maxima* in the Andaman Sea may occur on a local scale due to specific patterns of water circulation. The presence of genetic differentiation among populations within a relatively moderate geographical range suggests that conservation programmes need to be planned carefully in order to protect the existing genetic diversity. Individuals selected for culture broodstock, or individuals selected for transfer in restocking programmes should, if possible, be genetically typed and matched against the target population. The potential of using *T. maxima* as a model species for restocking programs of other giant clams need to be further investigated since behaviour seems to be flexible both within and between species of giant clams from the same area. More studies will be required to elucidate this issue.

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