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High genetic divergence of *Tridacna squamosa* living at the west and the east coasts of Thailand

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Starch-gel protein electrophoresis was employed to compare genetic divergence of giant clam, *Tridacna squamosa* populations from the Andaman Sea (23 individuals) and the Gulf of Thailand (47 individuals). The genetic data was obtained from 6 polymorphic enzyme loci. The allelic and genotypic frequencies between two populations were significantly different. The population genetic differentiation indices over all loci indicated high genetic divergence (Nei's genetic distance was 0.6212 and F_{st} 0.2148). Based on this result, the authors suggest that there should be no restocking of *T. squamosa* from the Andaman Sea to the Gulf of Thailand and vice versa, in order to maintain the genetic uniqueness of each population and prevent genetic incompatibility.

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INTRODUCTION

Giant clam is one of the endangered marine organisms being conserved in Thailand according to the list for protected species under Thai Law. Among three giant clam species in Thai waters, *Tridacna squamosa* made up less than 1% (recalculated from Chantrapornsyl *et al.* 1996; Banchongmanee & Upanoi 2000). However, the success on mass culture of *T. squamosa* at Prachuap Khiri Khan Coastal Aquaculture Development Center, Thailand (Nugranad *et al.* 1997) lights up a potential to restock this species to depleted natural populations. Restocking programmes have been carried out at several sites in the Gulf of Thailand, such as Tao Island and Man Islands (Nugranad *et al.* 1997; Nugranad *et al.* 1999; Charuchinda & Asawangkune 2000). Translocation of hatchery-produced clams from the Gulf of Thailand to some habitats in the Andaman Sea has been considered. These considerations led to the question if there is any genetic divergence between these two populations (Gulf of Thailand versus Andaman Sea). A mixing of two different intra-specific populations may lead to genetic incompatibility and finally decrease population size (Meffe & Carroll 1994). The worst scenario will be extinction of the species. In order to check this, we compared a population from the Andaman Sea with one from the Gulf of Thailand by means of isozymes.

MATERIALS AND METHODS

Samples collection

Mantle tissues of *Tridacna squamosa* were obtained from two places in Thailand: Surin

Islands, the Andaman Sea (23 individuals) and the Gulf of Thailand (47 individuals). The tissue samples from Surin Islands were collected during 6-10 April 2000, while the ones from the Gulf of Thailand were collected in July 1999 from broodstocks reared in Prachuap Khiri Khan Coastal Aquaculture Development Center since 1992. The mantle tissues were cut into 1-2 cm^2 pieces and stored in liquid nitrogen during collecting and transportation. The samples then were transferred to a –80 °C freezer for a month prior to an electrophoresis analysis.

Isozyme analysis

The tissue samples were added with 30-60 µl of 1 % polyvinyl pyrrolidone (PVP) and a little amount of cleaned sand before homogenizing. After centrifuged at 5,000 rpm for 5 minutes, the supernatants were absorbed onto paper wicks, which then were inserted into starch gels. Starch gels were prepared with 12% starch (Sigma S-4501) in tris citrate buffer pH 7.0 (Benzie 1993). The proteins were separated at 600 volt, 80 mA for 3 hours. Staining was done for 6 enzymes (Benzie *et al.* 1993) *i.e.* phophoglucomutase (PGM, EC 5.4.2.2), dihydrolipoamide dehydrogenase (DDH or diaphorase DIA, EC 1.8.1.4), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), malate dehydrogenase (MDH, EC 1.1.1.37), mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), peptidase using leucyl-glycyl-glycine (PEP-LGG, 3.4.X.X). The staining recipes were according to Harris & Hopkinson (1976) and Manchenko (1994).

Data analysis

The zymograms were scored for genotypes by assigning the fastest anodic-migrating allele as "1", the second fastest as "2", and so on. Testing of Hardy-Weinberg equilibrium with the H_1 hypotheses "heterozygote deficit" and "heterozygote excess" were carried out. Significant differences between populations were revealed by comparing allelic and genotypic frequencies. The magnitude of genetic divergence between two populations was calculated as Nei's genetic distance (Nei 1987) and F-statistics (Weir & Cockerham 1984). All calculated parameters were performed with computer programmes

Table 1. Allelic frequencies of *Tridacna squamosa* from Andaman Sea (A) and the Gulf of Thailand (G), N is the number of individuals typed.

Locus	Population	Allele							
		1	2	3	4	5	6	7	N
DDH	A	-	0.04	0.76		0.20	-	-	23
	G	-	0.20	0.46	0.32	0.02	-	-	47
GPI	A		0.02	0.14	0.80	0.05	-	-	22
	G	0.40	0.14	0.34	0.11	0.01	-	-	47
LGG	A	0.13	0.30	0.57	-	-	-	-	23
	G	0.31	0.55	0.10	0.04	-	-	-	47
MDHII	A	-	0.04	0.04	0.11	0.54	0.17	0.09	23
	G	0.15	0.02	0.38	0.06	0.17	0.11	0.11	43
MPI	A	0.08	0.34	0.26	0.32	-	-	-	19
	G	0.05	0.05	0.03	0.48	0.40	-	-	20
PGMII	A	-	0.15	0.48	0.09	0.28	-	-	23
	G	0.34	0.46	0.18	0.01	0.01	-	-	34

"Genepop version 3.2a" (Raymond & Rousset 1995) and "Popgene version 1.31" (Yeh *et al.* 1997).

RESULTS

The 6 stained enzymes revealed 8 loci (PGM and MDH had 2 loci while each of DDH, GPI, MPI and LGG had 1 locus). However, only 6 polymorphic loci were scored (PGMI had too pale bands and MDHI was monomorphic). All loci deviated significantly from Hardy-Weinberg equilibrium except DDH. All scored loci showed heterozygote deficit. Allelic frequencies of *T. squamosa* from the Gulf of Thailand and the Andaman Sea are shown in Table 1. There were significant differences in genotypic and allelic frequencies between two populations. Nei's genetic distance and F_{st} were 0.62 and 0.14 respectively when calculated with Popgene. Nevertheless, the F_{st} value, calculated with Genepop, gave different value (0.22).

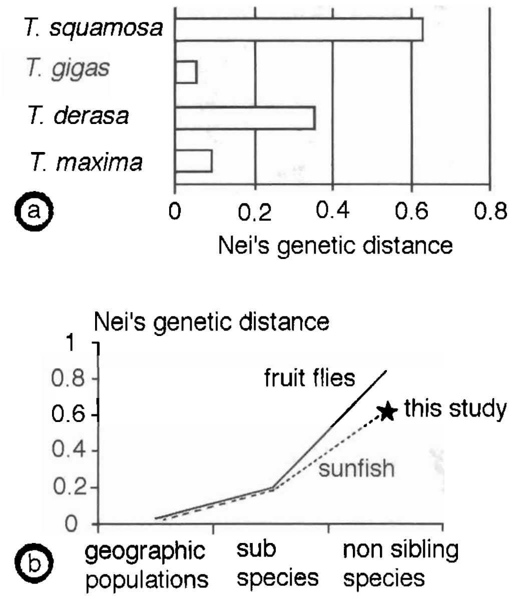

Figure 1. (a) Nei's genetic distance of *Tridacna squamosa* of the present study compared to data of *T. gigas* (Benzie & Williams 1992; Benzie & Williams 1995), *T. derasa* (Macaranas *et al.* 1992), and *T. maxima* (Benzie & Williams 1992; Kittiwattanawong 1997). (b) Avise (1994) used Nei's genetic distance to categorise geographic populations, sub species, and non-sibling species using data of complex fruit fries (*Drosophila willistoni*) and sunfish (*Lepomis*). Data of the present study are shown for comparison (see text for cautions).

DISCUSSION

Allelic and genotypic frequencies of *T. squamosa* in the Gulf of Thailand differed significantly from *T. squamosa* in the Andaman Sea. The magnitude of genetic divergence can be read from Nei's genetic distance and F_{st} values. Nei's genetic distance was higher than in other giant clam species: *T. gigas, T. derasa, T. maxima* (Fig. 1a). However, it may not mean that *T. squamosa* has higher divergence than the mentioned species because the value depends on many variables, such as biology and history of the animals, complexity of the habitats, geographic distance, and choice of enzymes. In addition, different algorithms employed in calculating software may give different results of the same data set. For instance in our study, F_{st} were different when calculated with Genepop version 3.2 ($F_{st} = 0.22$) and Popgene version 1.31 ($F_{st} = 0.14$).

Avise (1994) used the values of genetic distance to categorize geographic populations, sub species, and non-sibling species (Fig. 1b). In this study Nei's genetic distance revealed that the two populations of *T. squamosa* differed as much as different species. However, one should consider this with caution, since the values vary due to the factors mentioned above.

Why is divergence so high and when did it occur?

Based on recent topography of South East Asia (Fig. 2d), the first question may be clarified. Peninsular Thailand-Malaysia is a terrestrial barrier. The only connection between the Gulf of Thailand and the Andaman Sea is the narrow Strait of Malacca. Thus, the gene flow of the two populations is greatly obstructed. Sea level fluctuations during the period of the last glaciation should also be considered. Fig. 2a shows that 140,000 years ago there were two

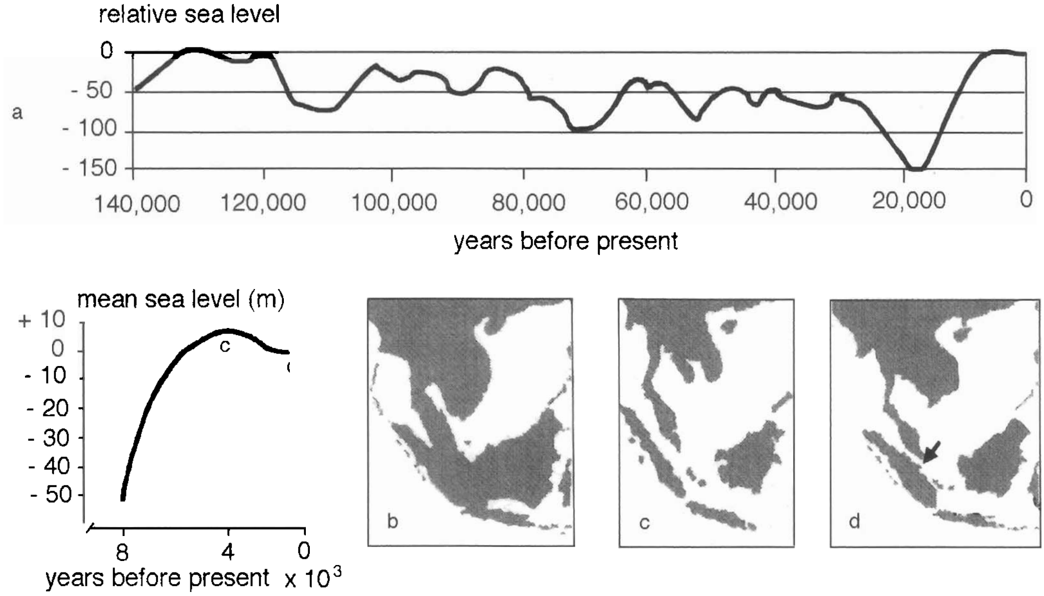

Figure 2. Sea level fluctuations (a) over the past 140,000 years (Sinsakul 1992). Sea level and topography of South East Asia: (b) 8000 years ago (c) 4000 years ago, (d) present situation (modified from Lekagul & McNeely 1977 and Geyh *et al.* 1979). Arrow (d) points at the Strait of Malacca.

periods with higher sea levels than at present. These periods with high sea level broadened the Strait of Malacca, and probably allowed a higher gene flow between the two bodies of water (Fig. 2c). In contrast, periods with lower sea level would narrow the seaway or even close it (Fig. 2d and 2b respectively).The divergence between *T. squamosa* from the Andaman Sea and the Gulf of Thailand might have happened from 4,000 to 130,000 years ago, or even more.

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