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# Genetic Diversity of the Giant Tiger Shrimp (*Penaeus monodon*) in Thailand Revealed by PCR-SSCP of Polymorphic EST-Derived Markers

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*A total of 90 ESTs from normal and 157 from subtractive ovarian cDNA libraries of the giant tiger shrimp* (Penaeus monodon) *were sequenced. SSCP analysis of* disulfide isomerase (DSl), zinc finger protein (ZFP), PMO920, *and* PMT1700 *was carried out for population genetic studies of* P. monodon *in Thai waters. The number of codominant alleles per locus for overall samples was 6 for* PMO920, *5 for* PMT1700, *and 12 for* ZFP, *and there were 19 dominant alleles for* DSI. *The observed heterozygosity of each geographic sample was 0.3043–0.5128 for* PMO920, *0.3462–0.4643 for* PMT1700, *and 0.5000–0.8108 for* ZFP. *Linkage disequilibrium analysis indicated that genotypes of these loci segregate randomly (*$P > 0.05$*). Low genetic distance was found between pairs of geographic samples (0.0077–0.0178). The neighbor-joining tree constructed from the average genetic distance of overall loci*

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*allocated the Andaman samples (Satun, Trang, and Phangnga) into one cluster, and Chumphon and Trat into other clusters. Geographic differentiation between Satun-Trat and Satun-Phangnga was found only at the* ZFP *locus* ($P < 0.05$)*, suggesting low degrees of genetic subdivision of Thai* P. monodon.

**KEY WORDS:** genetic diversity; SSCP; EST; *Penaeus monodon*; shrimp.

## INTRODUCTION

Thailand has been regarded as the leader for shrimp production (mainly *Penaeus monodon*) for over a decade. The annual production was approximately 280,000 metric tons in 2001. At present, the life cycle of *P. monodon* has not been closed in aquaculture hatcheries because breeding of *P. monodon* in captivity is extremely difficult. Accordingly, farming of *P. monodon* relies almost entirely on wild-caught broodstock for the supply of juveniles (Klinbunga *et al.*, 1999; Withyachumnarnkul *et al.*, 1998).

The open reproductive cycle and reliance on wild stocks of *P. monodon* results in heavy exploitation of female broodstock from natural populations (Klinbunga *et al.*, 1999). Presently, the government organization (Department of Fisheries) is concerned about actively enhancing natural *P. monodon* in Thai waters with hatchery-reared larvae. Some controls on the movement of live shrimps are now being considered because of the outbreak of viral and bacterial diseases.

The estimation and partition of the level of intraspecific genetic variation in any species is fundamental for establishing rational management of natural resources (Avise, 1994). In recent years, DNA analysis has been commonly used to determine the levels of intraspecific genetic variation and population differentiation of several shrimp species (Benzie, 2000; Benzie *et al.*, 2002; Maggioni *et al.*, 2001). Genetic markers are potentially useful for several applications in *P. monodon*, for instance, in the investigation of genetic variability of wild stocks, genetic improvement of domesticated stocks through selective breeding programs, maintaining genetic characteristics of an artificially propagated stock, and enhancement of local *P. monodon* stocks.

The objective of this study was to determine genetic diversity of *P. monodon* in Thai waters using polymorphism of coding nuclear DNA. The information obtained can be used for the construction of an appropriate stock enhancement program of *P. monodon* in Thailand and avoidance of the use of inbred founder stocks for domestication and selective breeding of *P. monodon*.

## MATERIALS AND METHODS

### Sampling

Broodstock-sized *P. monodon* used for construction of cDNA libraries were collected from Chonburi, located in the Gulf of Thailand. Specimens used for population genetic studies were broodstock caught alive from Chumphon ($N = 28$) and Trat ($N = 28$), located in the Gulf of Thailand, and Satun ($N = 39$), Trang ($N = 23$), and Phangnga ($N = 29$), located in the Andaman Sea (Fig. 1).

### Total DNA and RNA Isolation

Total RNA was extracted from immature and mature ovaries, and testes of broodstock-sized *P. monodon* using Tri-Reagent (Molecular Research Center). Messenger RNA was further purified using a QuickPrep Micro mRNA Purification Kit (GE Healthcare Bio-Sciences) and kept under absolute ethanol at -70°C until used.

Genomic DNA was extracted from a frozen pleopod of each shrimp using the SDS–phenol–chloroform method (Klinbunga *et al.*, 1996). Concentrations of extracted DNA and RNA were spectrophotometrically determined (Maniatis *et al.*, 1982).

### Construction of Normal and Subtractive cDNA Libraries

Purified mRNA (3 $\mu$g) of immature ovaries was reverse-transcribed to cDNA using a Superscript Plasmid System with Gateway Technology cDNA Synthesis and Cloning Kit (Invitrogen). Size-fractionated cDNA ( > 500 bp) was directionally ligated to a *Not*I/*Sal*I digested pSPORTl at 16°C for 16 h. Recombinant plasmids were transformed into *E. coli* DH5-α (Maniatis *et al.*, 1982). Sizes of inserts were verified by colony PCR using pUCl (5′-TCC GGC TCG TAT GTT GTG TGG A-3′) and pUC2 (5′-GTG GTG CAA GGC GAT TAA GTT GG-3′). Subtractive cDNA libraries of ovaries and testes of *P. monodon* were constructed using a PCR Select cDNA Subtraction Kit (Clonetech) as described in Leelatanawit *et al.* (2004).

In total, 90 positive colonies from the normal and 157 from the subtractive cDNA libraries (cDNA of ovaries as the tester) of *P. monodon* ovaries were randomly selected. Plasmid DNA was extracted using a GFX Micro Plasmid Prep Kit (GE Healthcare Bio-Sciences) and unidirectionally sequenced using a Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing Kit (GE Healthcare Bio-Sciences) with the M13 reverse primer on an automated DNA sequencer (LI-COR). Nucleotide sequences were compared with those deposited in GenBank using BLAST*N* and

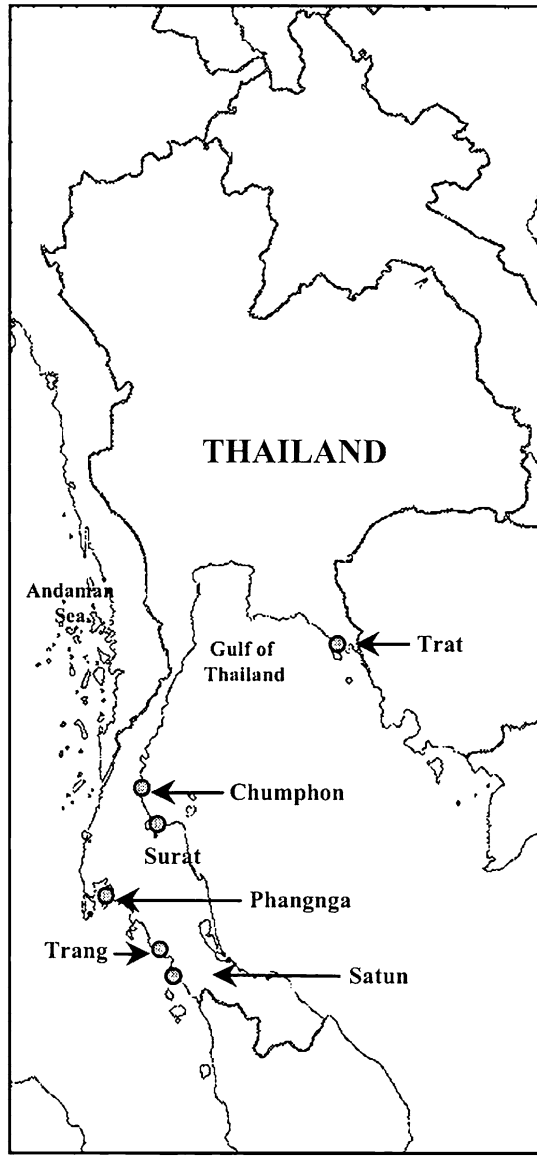


**Fig. 1.** Sampling sites for five geographic samples of *Penaeus monodon* in Thailand.

BLAST*X* (Altschul *et al*., 1990, available at http://www.ncbi.nlm.nih.gov). Significant probabilities were considered when E-values were $< 10^{-4}$.

## PCR of *DSI, ZFP, PMO920*, and *PMT1700*

Four genes were amplified by PCR. These included *DSI* (F: 5′-GCC GTT GCC AAT AAG GAC GA-3′ and R: 5′-TCA CCC GCC TTG AGA TTG GT-3′) and *ZFP* (F: 5′-TCG AAA CCC TTG CCG CAT AT-3′ and R: 5′-TGC TTG AAT CTC CCT CAT CCC-3′) found in the subtractive and normal cDNA libraries and *PMO920* (F: 5′-TTG AAC CAG TGT TTC TGC AAG T-3′ and R: 5′-TCG AAT AGC ACC AAG TCA TCA A-3′), which is an unknown transcript specifically expressed in ovaries, and *PMT1700* (F: 5′-CGT AAC CAG TAA GAG ATC GGG AG-3′ and R: 5′-GCT TTT TGG CAG TTT AAG AGA GTC-3′), which is an unknown transcript specifically expressed in testes of *P. monodon* (Leelatanawit *et al*., 2004).

PCR was carried out in a 50 $\mu$L reaction mixture containing 10 mM Tris–HCl, pH 8.8, 50 mM KCl, 0.1% Triton X-100, 2 mM of $MgCl_2$, 100 $\mu$M of each dNTP, 0.2 $\mu$M of each primer, 1 U of DyNazyme DNA polymerase (Finnzymes), and 25 ng of genomic DNA. The amplification reaction was initially performed by predenaturation at 94°C for 3 min followed by 35 cycles of a 94°C denaturation for 45 s, a 55°C annealing for 60 s, and a 72°C extension for 60 s. The final extension was carried out at 72°C for 7 min. Each amplification product (5 $\mu$L) was electrophoretically analyzed through 1.8% agarose gel, and visualized under a UV transilluminator after ethidium bromide staining (Maniatis *et al*., 1982).

## SSCP Analysis

The amplification product (6 $\mu$L) was mixed with four volumes of the loading dye (95% formamide, 0.25% bromophenol blue, 0.25% xylene cyanol, and 10 mM NaOH), denatured in a boiling bath for 5 min, and immediately cooled on ice for 3 min. The denatured products of *DSI* (native 12.5% + 5% glycerol PAGE; 37.5:1 crosslink), *ZFP* and *PMT1700* (native 15% PAGE; 75:1 crosslink), and *PMO920* (6% denaturing PAGE; 19:1 crosslink) were electrophoretically analyzed at 250–300 volts for 16–24 h at 4°C (SSCP) or 3 h (denaturing PAGE) at room temperature. Electrophoretic bands were visualized by silver staining.

## Data Analysis

For *PMO920*, *ZFP*, and *PMT1700*, observed and expected heterozygosity were calculated (Nei, 1987). The effective number of alleles at each locus was

examined (Crow and Kimura, 1965). Hardy–Weinberg expectations and genotypic disequilibrium were analyzed using the exact test. Geographic heterogeneity in allele distribution frequencies among compared samples was carried out with the Markov chain approach for $v^2$ analysis (Guo and Thompson, 1992). $F_{ST}$ between pairs of samples (Weir and Cockerham, 1984) was determined if it was significantly different from zero. All tests were routine in Genepop (Raymond and Rousset, 1995). The possible existence of a recent reduction of effective population sizes in each geographic sample was examined by Bottleneck (http://www.montpeller.inra.fr/URLB/bottleneck/pub.html).

For *DSI*, each fragment was treated as an independent character. Sizes of the bands were estimated by comparing with a 100 bp ladder and recorded in a binary matrix to represent the presence or absence of a particular band (Nei and Li, 1979).

The average genetic distance between geographic samples across overall loci was calculated and used to construct a neighbor-joining tree (Saitou and Nei, 1987) using Neighbor in Phylip (Felsenstein, 1993). Geographic heterogeneity and $F_{ST}$ statistics (h) between pairs of samples were analyzed using TFPGA (http://www.public.asu.edu/~mmille8/tfpga.htm).

## RESULTS

### Development of Molecular Markers

Recombinant clones of normal (90) and subtractive (157) cDNA of ovaries of *P. monodon* were sequenced. *Peritrophin*, *thrombospondin* (*TSP*), and unknown transcripts were abundantly expressed in both immature (Table I) and mature ovaries of *P. monodon* (Leelatanawit *et al*., 2004). Homologues of *zinc finger protein* (*ZFX*/*ZFY*, hereafter called *ZFP*) and *disulfide isomerase* (*DSI*) were found in these libraries.

RT-PCR using primers designed from conserved sequences of *DMRTI*/*dsx*/*Mab-3* (DM domain) generated fragments of approximately 920 bp (*PMO920*) and 1700 bp (*PMT1700*) specifically found in ovaries and testes of *P. monodon*, respectively. These fragments were regarded as unknown transcripts after sequencing and BLAST analysis.

### Genetic Diversity of Thai *P. monodon*

Codominant alleles were found at three loci: 6 (denaturing PAGE) at *PMO920*, 5 (SSCP) at *PMT1700*, and 12 (SSCP) at *ZFP*. Additionally, *DSI* (SSCP) generated 19 polymorphic fragments across investigated samples of *P. monodon* (Fig. 2). The average effective number of alleles for overall loci in Satun ($n_e$ = 2.238) and Trang (2.236) was slightly greater than that of

**Table I.** Summary of ESTs Isolated from Immature Ovaries of *Penaeus monodon*

| EST[a] | Closest species | *E*-value[b] | Redundancy |
|---|---|---|---|
| *Peritrophin* | *Penaeus monodon* | 0.0 | 30 |
| *Thrombospondin* | *Penaeus monodon* | 0.0 | 5 |
| *Clottable protein* | *Penaeus monodon* | $2\times10^{-132}$ | 3 |
| *Actin-related protein* | *Acanthaoeba castellanii* | $5\times10^{-32}$ | 1 |
| *Allergen Penm2* | *Penaeus monodon* | 0.0 | 1 |
| *Catalase* | *Campylobacter jejuni* | $1\times10^{-14}$ | 1 |
| *cDNA clone IMAGE:4393038* | *Homo sapiens* | $2\times10^{-12}$ | 1 |
| *CG6197-PA* | *Drosophila melanogaster* | $9\times10^{-5}$ | 1 |
| *Chaperonin 6A* | *Danio rerio* | $7\times10^{-10}$ | 1 |
| *Cortical rod protein* | *Marsupenaeus japonicus* | $8\times10^{-6}$ | 1 |
| *Elongation factor-α* | *Capsicum annuum* | $2\times10^{-6}$ | 1 |
| *Elongation factor-γ* | *Xenopus laevis* | $4\times10^{-4}$ | 1 |
| *Epimerase* | *Arabidopsis thaliana* | $6\times10^{-4}$ | 1 |
| *GTPase activating protein* | *Gallus gallus* | $9\times10^{-6}$ | 1 |
| *Histone* | *Litopenaeus vannamei* | $1\times10^{-16}$ | 1 |
| *Hypothetical protein* | *Lonomia obliqua* | $1\times10^{-5}$ | 1 |
| *Replication factor* C | *Homo sapiens* | $3\times10^{-8}$ | 1 |
| *RNA binding protein* | *Bombyx mori* | $4\times10^{-7}$ | 1 |
| *Ribosomal protein S20* | *Palaemonetes pugio* | $3\times10^{-31}$ | 1 |
| *Ribosomal protein S24* | *Marsupenaeus japonicus* | 0.0 | 1 |
| *Ribosomal protein L27a* | *Penaeus monodon* | $2\times10^{-58}$ | 1 |
| *Sentrin/SUMO-specific protease* | *Apis mellifera* | $1\times10^{-16}$ | 1 |
| *β-thymosin* | *Dermacentor variabilis* | $2\times10^{-25}$ | 1 |
| *Zinc finger protein* | *Homo sapiens* | $4\times10^{-12}$ | 1 |
| *Ubiquitin-conjugating enzyme E2L* | *Homo sapiens* | $8\times10^{-6}$ | 1 |
| *Unknown* | – | – | 30 |

[a] GenBank accession numbers DV738097-DV738186.
[b] The greatest *E*-value among redundant clones of an identical transcript was reported.

Chumphon (2.136) and Trat (2.057). The lowest $n_e$ was observed in *P. monodon* from Phangnga (2.013).

Relatively high genetic diversity was observed across all loci. The average $H_O$ of each geographic sample was comparable (0.5694 in Satun, 0.4348 in Trang, 0.5062 in Phangnga, 0.4762 in Chumphon, and 0.4500 in Trat; Table II). Allele frequencies of *DSI* were calculated from presence and absence of a particular amplification product. The number of polymorphic fragments (<95%) in each geographic sample at the *DSI* locus was 10, 8, 11, 9, and 10 alleles, respectively (Table III).

Considering loci exhibiting codominant segregation patterns, all geographic samples conformed to Hardy–Weinberg equilibrium at *PMT1700*. Deviation from Hardy–Weinberg expectation was observed in Trang ($P = 0.0002$) and Trat ($P = 0.0005$) at the *PMO920* locus due to homozygote excess, and in Satun ($P = 0.0047$) and Phangnga ($P = 0.0175$) at the *ZFP* locus due to heterozygote excess. A recent reduction of effective population size as a consequence of bottleneck effects was not found in any geographic sample

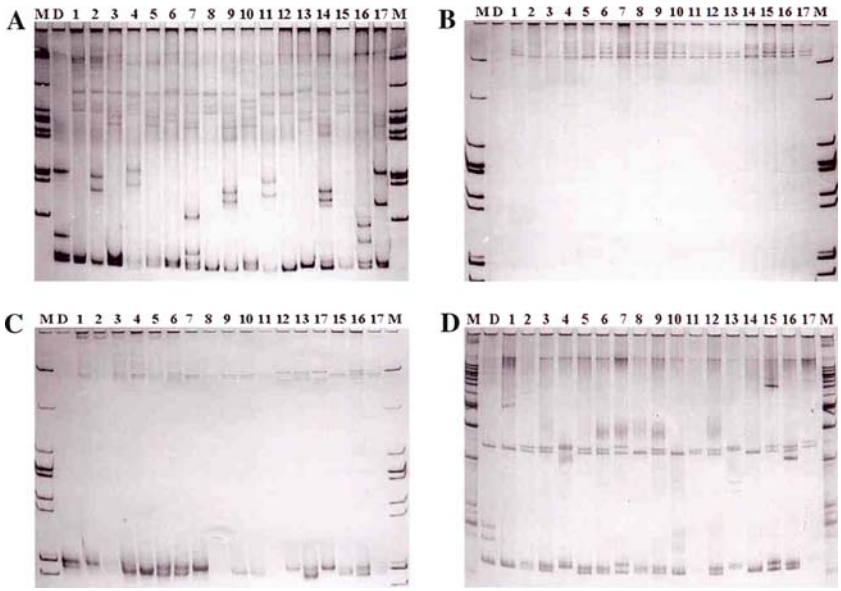


**Fig. 2.** SSCP patterns of the amplified *DSI* (A), *ZFP* (B), *PMT1700* (C), and *PMO920* (D) of *P. monodon* individuals (lanes 1–17) size-fractionated through nondenaturing (A, B, and C) and denaturing (D) polyacrylamide gels. Lanes M, 100 bp ladder, lanes D, nondenatured PCR product (double strand control).

($P > 0.05$). *PMO920*, *PMT1700*, and *ZFP* did not show genotypic disequilibrium for overall samples and within each geographic sample ($P > 0.05$).

The average genetic distance between geographic samples was 0.0031 (Satun-Trang) to 0.0178 (Trang-Chumphon). Genetic distances between samples from the different coastal areas were 0.0079 (Phangnga-Trat) to 0.0178 (Chumphon-Trang), whereas the distances within the Gulf of Thailand and the Andaman samples were 0.0077 and 0.0031–0.0135, respectively (Table IV).

The neighbor-joining tree constructed from genetic distance between pairs of geographic samples of *P. monodon* indicated separate phylogenetic groups according to coastal origins (the Andaman Sea and the Gulf of Thailand) of investigated samples (Fig. 3).

Geographic heterogeneity analysis using the exact test and $F_{ST}$ statistics indicated that population differentiation was not significant in almost all of the samples at the *PMO920*, *PMT1700*, *ZFP*, and *DSI* loci ($P > 0.05$), except between Satun-Phangnga ($P < 0.0307$) and Satun-Trat ($P < 0.0092$) at the *ZFP* locus (Table V). The probability value calculated from $F_{ST}$ statistics between Satun and Chumphon at this locus was marginally not significant ($P = 0.0563$).

**Table II.** Allele Frequencies at Three Loci of *P. monodon* from Five Locations in Thailand

| | Allele frequency | | | | |
|---|---|---|---|---|---|
| Locus/allele | Satun | Trang | Phangnga | Chumphon | Trat |
| *PMO920* | | | | | |
| 1 | 0.1410 | 0.1739 | 0.1034 | 0.0714 | 0.1250 |
| 2 | 0.5897 | 0.6087 | 0.6034 | 0.6071 | 0.5536 |
| 3 | 0.0513 | 0.0435 | 0.1724 | 0.0357 | 0.0714 |
| 4 | 0.2051 | 0.1522 | 0.1034 | 0.2143 | 0.1786 |
| 5 | 0.0128 | 0.0217 | 0.0172 | 0.0357 | 0.0357 |
| 6 | 0.0000 | 0.0000 | 0.0000 | 0.0357 | 0.0357 |
| $H_O$ | 0.5128 | 0.3043 | 0.4827 | 0.5000 | 0.3571 |
| $H_e$ | 0.5951 | 0.5865 | 0.5947 | 0.5870 | 0.6500 |
| HW (*P*-value) | 0.1209 | 0.0002* | 0.2455 | 0.3276 | 0.0005* |
| *N* | 39 | 23 | 29 | 28 | 28 |
| *PMT1700* | | | | | |
| 1 | 0.7436 | 0.7609 | 0.8077 | 0.6429 | 0.7321 |
| 2 | 0.2436 | 0.1957 | 0.1731 | 0.3571 | 0.2500 |
| 3 | 0.0128 | 0.0217 | 0.0000 | 0.0000 | 0.0000 |
| 4 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0179 |
| 5 | 0.0000 | 0.0217 | 0.0192 | 0.0000 | 0.0000 |
| $H_O$ | 0.3846 | 0.3478 | 0.3462 | 0.4286 | 0.4643 |
| $H_e$ | 0.3926 | 0.3903 | 0.3235 | 0.4675 | 0.4084 |
| HW (*P*-value) | 0.4744 | 0.1789 | 0.6562 | 0.4800 | 0.8868 |
| *N* | 39 | 23 | 28 | 28 | 28 |
| *ZFP* | | | | | |
| 1 | 0.4865 | 0.5000 | 0.6379 | 0.6429 | 0.6923 |
| 2 | 0.3919 | 0.3696 | 0.2759 | 0.2500 | 0.2500 |
| 3 | 0.0405 | 0.0000 | 0.0000 | 0.0536 | 0.0000 |
| 4 | 0.0000 | 0.0000 | 0.0172 | 0.0000 | 0.0000 |
| 5 | 0.0000 | 0.0217 | 0.0000 | 0.0179 | 0.0385 |
| 6 | 0.0135 | 0.0217 | 0.0517 | 0.0179 | 0.0000 |
| 7 | 0.0135 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 8 | 0.0000 | 0.0435 | 0.0172 | 0.0000 | 0.0192 |
| 9 | 0.0135 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 10 | 0.0270 | 0.0000 | 0.0000 | 0.0179 | 0.0000 |
| 11 | 0.0135 | 0.0217 | 0.0000 | 0.0000 | 0.0000 |
| 12 | 0.0000 | 0.0217 | 0.0000 | 0.0000 | 0.0000 |
| $H_O$ | 0.8108 | 0.6522 | 0.6897 | 0.5000 | 0.5385 |
| $H_e$ | 0.6150 | 0.6232 | 0.5227 | 0.5299 | 0.4653 |
| HW (*P*-value) | 0.0047** | 0.6531[a] | 0.0175** | 0.5641[a] | 0.9038[a] |
| *N* | 37 | 23 | 29 | 28 | 26 |

HW, Hardy–Weinberg disequilibrium analysis; *, significant at $P < 0.05$; **, heterozygote excess test and significant at $P < 0.05$. [a], heterozygote deficiency test.

## DISCUSSION

Population genetic studies of *P. monodon* have been reported based on PCR-RFLP of mitochondrial DNA (Klinbunga *et al.*, 1999, 2001; Benzie *et al.*, 2002), RAPD (Tassanakajon *et al.*, 1998), and type II microsatellites

**Table III.** Allele Frequencies of *P. monodon* from Five Locations in Thailand Based on SSCP Analysis of *DSI*

| Band[a]/Sample | Satun | Trang | Phangnga | Chumphon | Trat |
|---|---|---|---|---|---|
| Band 1 | 0.0132 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Band 2 | 0.0000 | 0.0000 | 0.0351 | 0.0364 | 0.0174 |
| Band 3 | 0.0000 | 0.0445 | 0.0351 | 0.0364 | 0.0174 |
| Band 4 | 0.5133 | 0.5337 | 0.5451 | 0.5371 | 0.5451 |
| Band 5 | 0.0823 | 0.0675 | 0.0351 | 0.0364 | 0.0531 |
| Band 6 | 0.0000 | 0.0220 | 0.0351 | 0.0000 | 0.0351 |
| Band 7 | 0.0681 | 0.0220 | 0.1490 | 0.1136 | 0.0903 |
| Band 8 | 0.4380 | 0.4102 | 0.4128 | 0.4024 | 0.5087 |
| Band 9 | 0.0968 | 0.1924 | 0.1290 | 0.1762 | 0.0531 |
| Band 10 | 0.0000 | 0.0000 | 0.0174 | 0.0364 | 0.0000 |
| Band 11 | 0.1115 | 0.1153 | 0.1094 | 0.0937 | 0.1490 |
| Band 12 | 0.1571 | 0.1153 | 0.0903 | 0.0742 | 0.1906 |
| Band 13 | 0.0823 | 0.0675 | 0.0715 | 0.1136 | 0.0715 |
| Band 14 | 0.0541 | 0.0445 | 0.1290 | 0.0551 | 0.1094 |
| Band 15 | 0.0000 | 0.0000 | 0.0351 | 0.0000 | 0.0000 |
| Band 16 | 0.2053 | 0.1403 | 0.1094 | 0.1136 | 0.2122 |
| Band 17 | 0.0132 | 0.0220 | 0.0531 | 0.0364 | 0.0174 |
| Band 18 | 0.0132 | 0.0220 | 0.0715 | 0.0180 | 0.0351 |
| Band 19 | 0.0132 | 0.0000 | 0.0174 | 0.0180 | 0.0000 |

[a] *DSI* was regarded as the diploid/dominant marker. As a result, the presence and absence of each band was scored.

**Table IV.** Pairwise Genetic Distance between Five Geographic Samples of *P. monodon* from Thailand

| | Satun | Trang | Phangnga | Chumphon | Trat |
|---|---|---|---|---|---|
| Satun | – | | | | |
| Trang | 0.0031 | – | | | |
| Phangnga | 0.0135 | 0.0102 | – | | |
| Chumphon | 0.0144 | 0.0178 | 0.0169 | – | |
| Trat | 0.0129 | 0.0129 | 0.0079 | 0.0077 | – |

Note: Analyzed by *PMO920, PMT1700, ZFP*, and *DSI* polymorphism.

(Supungul *et al*., 2000; Xu *et al*., 2001). However, information based on the type I markers (coding sequences) on population genetics of *P. monodon* has not been reported.

Four EST-derived markers (*PMO920*, *PMT1700*, *ZFP*, and *DSI*) were applied for examining genetic diversity and population subdivisions of *P. monodon* in Thai waters. Initial screening of these genes using representative individuals of *P. monodon* by agarose gel electrophoresis revealed both homozygotic and heterozygotic states for *PMO920*, whereas the amplification products of the remaining genes did not show allelic variations or indels between investigated individuals. As a result, genotypes of *P. monodon* at *PMO920* were analyzed by denaturing polyacrylamide gel electrophoresis,

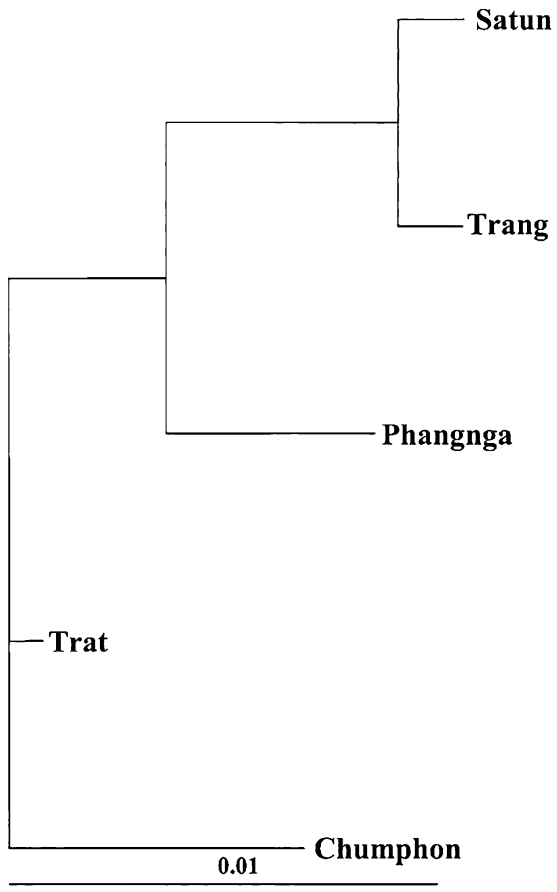


**Fig. 3.** A neighbor-joining tree indicating genetic relationships between conspecific samples of *P. monodon* analyzed by polymorphism of *PMO920, PMT1700, ZFP*, and *DSI*.

whereas those of *DSI*, *PMT1700*, and *ZFP* were analyzed by SSCP analysis. Results indicated complicated band patterns of the amplified *DSI*, and SSCP bands of this gene were treated as dominant markers, whereas those of the remaining genes were scored as codominant markers.

No genotypic disequilibrium was observed, indicating that genotypes of *PMO920*, *PMT1700*, and *ZFP* did not associate nonrandomly. Significant deviation from the Hardy–Weinberg expectation was found only in 4 of 15 possible tests ($P < 0.05$).

The deficiency of fixed population- or region-specific genotypes and the presence of shared genotypes with relatively comparable allele frequencies across geographic samples were found. This implied weak genetic

**Table V.** Pairwise Comparisons of Genetic Differentiation between Conspecific Samples of *P. monodon*

| Geographic Sample | Geographic Heterogeneity Test (*P*-value) | | | | $F_{ST}$ (*P*-value) | | | |
|---|---|---|---|---|---|---|---|---|
| | *PMO920* | *PMT1700* | *ZFP* | *DSI* | *PMO920* | *PMT1700* | *ZFP* | *DSI* |
| SAT-TNG | 0.9420 | 0.5719 | 0.3575 | 1.0000 | 0.9222 | 0.7659 | 0.8043 | > 0.05 |
| SAT-PHA | 0.0963 | 0.4193 | 0.1006 | 0.6808 | 0.2682 | 0.3823 | **0.0307*** | > 0.05 |
| SAT-CHM | 0.4460 | 0.2398 | 0.4757 | 0.9811 | 0.4757 | 0.2320 | 0.0563 | > 0.05 |
| SAT-TRT | 0.6091 | 0.8067 | **0.0336*** | 0.9998 | 0.9287 | 1.0000 | **0.0092*** | > 0.05 |
| TNG-PHA | 0.2311 | 0.8455 | 0.3738 | 0.9984 | 0.4827 | 0.7886 | 0.1479 | > 0.05 |
| TNG-CHM | 0.5171 | 0.0888 | 0.1470 | 1.0000 | 0.6418 | 0.1281 | 0.1491 | > 0.05 |
| TNG-TRT | 0.8346 | 0.4993 | 0.2840 | 0.9984 | 0.9048 | 0.6585 | 0.0745 | > 0.05 |
| PHA-CHM | 0.0565 | 0.0397 | 0.3335 | 1.0000 | 0.2278 | 0.0515 | 0.7235 | > 0.05 |
| PHA-TRT | 0.3041 | 0.3792 | 0.3352 | 0.9999 | 0.5528 | 0.3241 | 0.5100 | > 0.05 |
| CHM-TRT | 0.8916 | 0.3052 | 0.5176 | 0.9239 | 0.8872 | 0.3042 | 0.6826 | > 0.05 |

*Note:* Four loci (*PMO920, PMT1700, ZFP*, and *DSI*) were compared using geographic heterogeneity (exact test) and $F_{ST}$ analyses.
*significant at $P < 0.05$.

differentiation between *P. monodon* from different geographic origins in Thai waters. A low level of genetic difference was observed between pairs of conspecific samples of Thai *P. monodon* (0.0031 between Satun and Trang to 0.0178 between Chumphon and Trang).

Supungul *et al.* (2000) examined genetic diversity of *P. monodon* in Thailand by microsatellites (*CUPmo1*, *CUPmo18*, *Di25*, *CSCUPmo1*, and *CSCUPmo2*) using the same sample set as in this study. The average observed heterozygosity was relatively high in each geographic sample (0.71–0.82). Significant deviation from the Hardy–Weinberg expectation was observed in 19 of 25 possible tests owing to homozygote excess, even after the sequential Bonferroni procedure (Rice, 1989) was applied for multiple tests ($P < 0.001$). However, Mendelian segregation was confirmed at all five loci using pedigree samples, and results showed the absence of nonamplifying (null) alleles at those loci ($P > 0.05$, data not shown). The greatest genetic distance was observed between Trat and Satun ($d = 0.030$), and the shortest distance was between Satun and Trang (0.024). Three microsatellite loci (*Di27, CSCUPmo1*, and *CSCUPmo2*) did not show any significant geographic differences among all pairwise comparisons. $F_{ST}$ between pairs of geographic samples was usually $< 0.01$. Low degrees of differentiation were observed between shrimps from Satun and Trat ($F_{ST} = 0.0124$, $P < 0.01$), and Trat and Chumphon ($F_{ST} = 0.0118$, $P < 0.01$).

Likewise, high diversity but low genetic differentiation was also reported in wild (Palawan, Quezon, Capiz, and Negros Occidental-W) and cultured (Negros Occidental-C and Antique) *P. monodon* in the Philippines

analyzed by six microsatellites (*TUZXPm*2.41, *TUZXPm*4.45, *TUZXPm*4.55, *TUZXPm*4.82, *TUZXPm*4.85, and *TUZXPm*4.9). The observed heterozygosity of these samples was 0.47–1.00. A low degree of population differentiation was found between Negros Occidental-W and other samples ($F_{ST}$ = 0.009–0.013), but not between the remaining pairwise comparisons ($F_{ST}$ = 0.000–0.001) (Xu *et al*., 2001).

Genetic diversity of Thai *P. monodon* was also reported based on PCR-RFLP of *16S rDNA* and *COI-COII*. A total of 37 mtDNA composite haplotypes were observed. High haplotype diversity (0.855) and nucleotide diversity (3.328%) of *P. monodon* were found in mtDNA analysis. Large genetic distance between geographic samples was observed, with the lowest distance of 0.000 between each of the Andaman samples and the greatest distance of 0.217 between Satun and Trat. Strong population differentiation of *P. monodon* between the Andaman Sea and the Gulf of Thailand was clearly illustrated ($P < 0.0001$; Klinbunga *et al*., 2001).

The failure to detect genetic heterogeneity in Thai *P. monodon* (except between Satun and Trat at the *ZFP* locus) by EST-derived markers may result from the use of a lower polymorphic level of type I markers (cDNA) than that of microsatellites and mtDNA gene segments against mixed populations of *P. monodon*. The contradiction between strong (mtDNA) and weak (microsatellites and EST-derived markers) genetic differentiation of the same sample set of *P. monodon* opens the possibility of nonequivalent gene flow levels between genders detected by mtDNA polymorphism (female gene flow) and microsatellites and EST-derived markers (gene flow resulting from both males and females).

Molecular population genetic studies provide information necessary for elevating culture and management efficiency of *P. monodon* (Carvalho and Hauser, 1994; Ward and Grewe, 1994). In the present study, we illustrate the potential of SSCP analysis of EST-derived markers for evaluation of genetic diversity of Thai *P. monodon*. The basic knowledge of levels of genetic diversity and population differentiation of *P. monodon* not only yields critical information on historical and evolutionary aspects of *P. monodon* but also allows the construction of effective breeding programs and stock enhancement projects in this species.

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