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Genetic Heterogeneity of the Tropical Abalone (*Haliotis asinina*) Revealed by RAPD and Microsatellite Analyses

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Genetic heterogeneity of the tropical abalone, *Haliotis asinina* was examined using randomly amplified polymorphic DNA (RAPD) and microsatellite analyses. One hundred and thirteen polymorphic RAPD fragments were generated. The percentage of polymorphic bands of *H. asinina* across overall primers was 85.20%. The average genetic distance of natural samples within the Gulf of Thailand (HACAME and HASAME) was 0.0219. Larger distance was observed when those samples were compared with HATRAW from the Andaman Sea (0.2309 and 0.2314). Geographic heterogeneity and F_{ST} analyses revealed population differentiation between *H. asinina* from the Gulf of Thailand and the Andaman Sea ($p < 0.0001$). Three microsatellite loci (*CUHas1*, *CUHas4* and *CUHas5*) indicated relatively high genetic diversity in *H. asinina* (total number of alleles = 26, 5, 23 and observed heterozygosity = 0.84, 0.42 and 0.33, respectively). Significant population differentiation was also found between samples from different coastal regions ($p < 0.0001$). Therefore, the gene pool of natural *H. asinina* in coastal Thai waters can be genetically divided to 2 different populations; the Gulf of Thailand (A) and the Andaman Sea (B).

Keywords: Abalone, Genetic diversity, Microsatellites, Population differentiation, RAPD

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Introduction

Abalone are economically important marine gastropods distributed throughout the tropical and the temperate zones (Geiger, 2000). Approximately 100 species of abalone are existent and over 15 species are farmed and commercially important (Jarayabhand and Paphavasit, 1996). The total world production of abalone was approximately 13,000 metric tons in 1999. Of these, 7,165 metric tons were from aquaculture where China (mainly *Haliotis discus hannai*) and Taiwan (mainly *H. diversicolor supertexta*) contributed approximately 75% of the cultured production annually (Chen *et al.*, 1989; Gordon, 2000). Three species of tropical abalone, *H. asinina*, *H. ovina* and *H. varia* are indigenously found in Thai waters. While *H. asinina* and *H. ovina* are found along the coasts of the upper Gulf of Thailand, all three species are distributed in the Andaman Sea (Nateewathana and Bussarawit, 1988). Among these species, *H. asinina* provides the highest percentage of a ratio between the meat weight and the total weight (85%) when compared to *H. varia* (30%) and *H. ovina* (40%) (Singhagraiwan and Doi, 1993). More importantly, *H. asinina* is a year-round spawning species. The spawning cycle is highly predictable. Therefore, *H. asinina* is the most promising species for the culture industry (Selvamani *et al*., 2001).

Relatively little is known about the basic knowledge on genetic diversity and population structure of *H. asinina*. This information is essential for the construction of an appropriate management scheme of *H. asinina* in Thailand (Jarayabhand *et al*., 2002). Appropriate genetic markers can be used to elevate the culture and management efficiency of abalone in Thailand. Sustainable aquaculture of *H. asinina* requires the basic knowledge on stock structure and the use of suitable molecular genetic markers to establish appropriate broodstock

management programmes and the effective breeding programmes to avoid significant reduction of genetic diversity of the cultured stocks (Klinbunga *et al.*, 2003).

Molecular genetic studies have been carried out in various abalone species (Brown, 1991, Metz *et al.*, 1998; Huang and Hana, 1998; Kirby *et al.*, 1998; Sweijd *et al.*, 1998; Huang *et al.*, 2000; Sekino and Hara, 2001) but publications concerning population genetics of *H. asinina* were rather limited. Recently, 11 microsatellite loci were isolated and characterised from the Heron reef sample of *H. asinina* (Selvamani *et al.*, 2000). Five of these (Has2k, Has2J, Has3K, Has10 and Has13) were used for genotyping of larvae produced by 3 separate crosses to assess fertilization success and maintaining pedigree information for selective breeding. The parents of an individual veliger could be determined from only 3 loci (Selvamani *et al.* 2001).

Genetic diversity of abalone in Thai waters; *H. asinina*, *H. ovina*, and *H. varia* were recently reported. Restriction analysis of 18S (nuclear) rDNA with *Alu* I, *Taq* I and *Hae* III and 16S (mitochondrial) rDNA with *Bam* HI, *Eco* RI, *Hae* III and *Alu* I gave 12 and 13 digestion patterns, respectively. A total of 49 composite haplotypes were found. Geographic heterogeneity analysis and F_{ST} estimate indicated strong genetic differentiation in *H. ovina* ($p < 0.0001$) but did not reveal genetic heterogeneity of *H. asinina* and *H. varia* in Thai waters ($p > 0.0021$) (Klinbunga *et al.* 2003).

The objectives of this study were analysis of genetic diversity and population differentiation levels of *H. asinina* using RAPD and microsatellite analyses. The basic knowledge obtained is useful to avoid including inbred founder populations and to design appropriate breeding scheme in breeding programmes of *H. asinina*. The information can also be applied for management of wild broodstock to ensure sustainable aquaculture of *H. asinina*.

Materials and Methods

Sampling The tropical abalone (*H. asinina*) were collected from 6 samples (Fig. 1) including natural abalone from Talibong Island located in Trang Province (HATRAW, $N = 28$), Samet Island located in Rayong Province (HASAME, $N = 19$) and Cambodia (HACAME, $N = 23$) (Fig. 1) and hatchery samples composing of the first (P_0) and the second (F_1) generation samples which founders were originated from Cambodia (HACAMHE, $N = 15$) and Samet Island (HASAMHE, $N = 15$) and from the Philippines (HAPHIE, $N = 30$), respectively.

DNA extraction Genomic DNA was extracted from a piece of the foot tissue of each *H. asinina* using a phenol/chloroform-proteinase K method (Klinbunga *et al.*, 2004). The concentration of extracted DNA was spectrophotometrically measured. DNA was kept at 4ºC until required.

RAPD analysis One hundred and thirty decanucleotide primers were screened (Fritsch *et al.*, 1993). Five selected primers (OPB11, 5'-GTAGACCCGT-3'; UBC101, 5'-GCGCCTGGAG-3'; UBC 195, 5'-GATCTCAGCG-3'; UBC197, 5'-TCCCCGTTCC-3' and UBC 271, 5'-GCCATCAAGA-3'), were used for population genetic analysis of *H. asinina.* PCR was carried out in a 25 ml reaction volume containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin, 100 mM of each dNTP, 0.2 mM of an arbitrary primer, 25 ng of genomic DNA and 1 unit of Ampli*Taq* DNA polymerase (Welsh and McClelland, 1990; Williams *et al.*, 1990). Amplification was carried out in a PCR Sprint thermalcycler (Hybaid, UK) consisting of a predenaturation at 94ºC for 3 minutes followed by 35 cycles of 94ºC for 15 seconds; 36ºC for 60 seconds and 72ºC for 90 seconds. The final extension was performed at 72ºC for 7 minutes. The amplification products were electrophoretically analysed through 1.6% gels and visualised under a UV transilluminator after ethidium bromide staining (Maniatis *et al.*, 1982).

Fig. 1. Map of Thailand indicating sample collection sites of *H. asinina* used in this study. Dots represent geographic locations (excluding the Philippines sample) from which *H. asinina* was collected. Samet Island (SAM) and Talibong Island are located in Rayong and Trang provinces, respectively.

Microsatellite analysis Three microsatellite loci; *CUHas1* possessed $(GT)_{17}N_{36}(GT)_{10}$, *CUHas4* possessed $(GT)_6(TGCA)_4N_{15}(GT)_7$ and *CUHas5* possessed $(GT)_{17}$, previously isolated in our laboratory (GenBank accession numbers BV096866, BV096869 and BV096864; Tang *et al.*, 2004) were used for population genetic studies of natural (HACAME and HATRAW) and hatchery samples of *H. asinina* (HACAMHE and HASAMHE). Genotyping of each abalone was performed only a single time for each locus. PCR was carried out in a 5 µl reaction volume containing 10 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 200 µM of each dNTP, 0.45 µM of the reverse primer, 0.425 µM of the forward primer, 0.025 µM of the [γ-^{32}P]dATP labelled forward

Table 1. Sequences of RAPD primers, size ranges, number of amplified bands and the percentage of polymorphic and monomorphic bands resulted from RAPD analysis of *H. asinina*

Primer	Sequence	Size-range (bp)	No. of bands	Polymorphic bands (%)	Monomorphic bands (%)
OPB11	5'-GTAGACCCGT-3'	390-2300	21	90.48	9.52
UBC101	5'-GCGCCTGGAG-3'	325-1800	32	81.25	18.75
UBC 195	5'-GATCTCAGCG-3'	525-1500	25	84.00	16.00
UBC197	5'-TCCCCGTTCC-3'	500-1500	23	86.96	13.04
UBC 271	5'-GCCATCAAGA-3'	250-1050	12	83.33	16.67
Total		250-2300	113	85.20	14.80

primer, 0.15 unit of DyNAzyme™ DNA polymerase and 25 ng of DNA template (or 1 µl of DNA extracted by a Chelex-based method; Walsh *et al.*, 1991). The reaction mixture was overlaid with a drop of mineral oil.

Amplification was performed in an Omnigene thermalcycler (Hybaid, UK) consisting of a predenaturation at 95ºC for 3 min followed by 30 cycles of denaturation at 95ºC for 1 min, annealing at 53ºC (*CUHas1*), 57ºC (*CUHas4*) or 49ºC (*CUHas5*) for 2 min and extension at 72ºC for 1 min. The final extension was carried out at 72ºC for 10 min. The PCR product was size-fractionated through 6% denaturing polyacrylamide gels. Sizes of microsatellite alleles were determined by comparing with the M13 sequencing marker after autoradiography (Yanish-Perron *et al.*, 1985).

Data analysis Each RAPD fragment is treated as an independent character. Sizes of RAPD bands were estimated by comparing with a 100 bp ladder and recorded in a binary matrix to represent the presence (1) or absence (2) of a particular band. The similarity index between individuals, within samples and between samples were calculated (Nei and Li, 1979). Genetic distance between paired samples was then estimated (Lynch, 1990) and used to construct a neighbor-joining tree (Saitou and Nei, 1987) using Neighbor in PHYLIP (Felsenstein, 1993). Geographic heterogeneity and F_{ST} statistics between pairs of samples were analysed using TFPGA (available at http://www.public.asu.edu/~mmille8/tfpga.htm).

For microsatellite analysis, 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 analysed using the exact test (Guo and Thompson, 1992). Geographic heterogeneity in allele distribution frequencies among compared samples was carried out with the Markov chain approach for χ^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 significance levels for multiple tests were adjusted following a sequential Bonferroni approach (Rice, 1989).

Results

RAPD analysis High genetic diversity of *H. asinina* was found from RAPD analysis. One hundred and thirteen RAPD fragments ranging from 250 bp to 2300 bp in length were generated across all investigated samples (21, 32, 25, 23 and 12 bands for OPB11, UBC101, UBC195, UBC197 and UBC271, respectively; Table 1). The percentage of polymorphic bands across overall samples generated from OPB11 was 90.48% whereas that of the remaining primer were 81.25-86.96%. Several RAPD bands including 1700 bp (UBC101, Fig. 2), 1025 bp and 650 bp (UBC195), 1400 bp and 710 bp (UBC197) and 650 bp (UBC271) bands were found in all investigated individuals and regarded as potential species-specific RAPD markers for *H. asinina*.

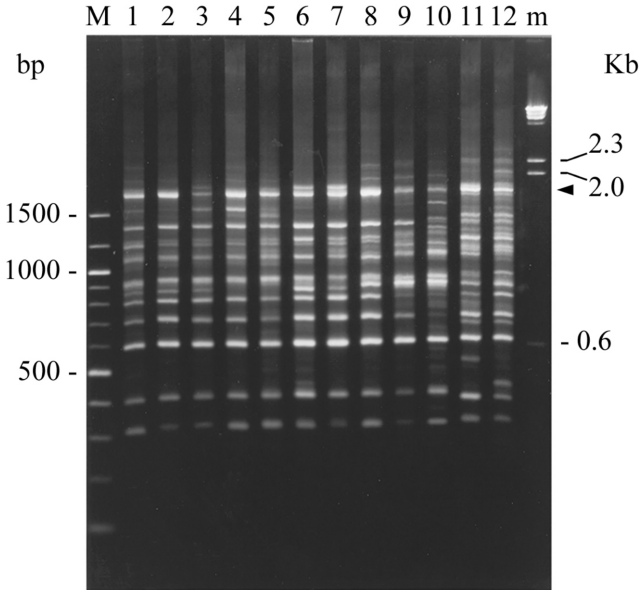

Fig. 2. Examples of RAPD patterns resulted from analysis of *H. asinina* from Samet Island (HASAME, lanes 1-4), Cambodia (HACAME, lanes 5-8), Talibong Island (HATRAW, lanes 9-10), and the Philippines (HAPHIE, lanes 11-12) with UBC101. Lanes M and m are a 100 bp ladder and λ-*Hin*d III, respectively. An arrowhead indicated a *H. asinina*-specific RAPD marker (1700 bp).

The similarity index within samples across overall primers of *H. asinina* was 0.7927 (HASAME)-0.8496 (HATRAW). Low genetic differences were found between pairs of the Gulf of Thailand samples (0.0156-0.0317) whereas greater genetic distance was found between samples from the Gulf of Thailand and HATRAW (0.2096-0.2381) and HAPHIE

Table 2. The average genetic distance (below diagonal) and similarity indices (above diagonal) between samples of *H. asinina* resulted from RAPD analysis using OPB11, UBC101, UBC195, UBC197 and UBC271

	HASAMHE	HASAME	HACAMHE	HACAME	HATRAW	HAPHIE
HASAMHE	-	0.9730	0.9737	0.9844	0.7619	0.7781
HASAME	0.0270	-	0.9683	0.9781	0.7686	0.7871
HACAMHE	0.0263	0.0317	-	0.9766	0.7904	0.7764
HACAME	0.0156	0.0219	0.0234	-	0.7691	0.7933
HATRAW	0.2381	0.2314	0.2096	0.2309	-	0.8203
HAPHIE	0.2219	0.2129	0.2236	0.2067	0.1797	-

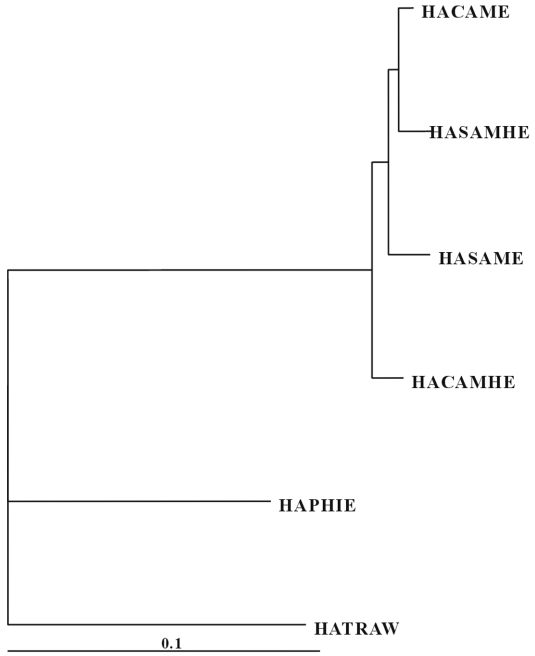

Fig. 3. A neighbor-joining tree illustrating genetic relationships of *H. asinina* based on the average genetic distance resulted from RAPD analysis using OPB11, UBC101, UBC195, UBC197, and UBC271.

(0.2067-0.2236) (Table 2).

Geographic heterogeneity analysis did not revealed genetic differentiation between *H. asinina* within the Gulf of Thailand ($p > 0.0083$ following a sequential Bonferroni method) but all RAPD primers showed significant heterogeneity between the Gulf of Thailand (HACAME, HACAMHE, HASAME and HASAMHE), the Andaman (HATRAW) and the Philippines (HAPHIE) samples ($p < 0.0001$). A neighbor-joining tree constructed from genetic distance between pairs of *H. asinina* samples also indicated large genetic differences among those samples (Fig. 3).

Microsatellite analysis Allele distribution patterns at *CUHas1*, *CUHas4* and *CUHas5* varied greatly among loci (Fig. 4 and Table 3). At the locus *CUHas1*, a 316 bp allele showed the greatest allele frequency in the Gulf of Thailand samples (0.158-0.300) but this allele was not found in HATRAW. At the locus *CUHas4*, 3 alleles (222, 224 and 228 bp) were found in HATRAW with comparable frequencies (0.450, 0.225 and 0.325, respectively) whereas 222 bp and 228 bp alleles were predominant in HASAMHE (0.600) and HACAME (0.864) and HACAMHE (0.550), respectively. At the locus *CUHas5*, 114 bp (0.150-0.214) and 154 bp (0.150-0.250) alleles were predominant in the Gulf of Thailand samples whereas 109 bp (0.482) and 110 bp (0.304) alleles were common in HATRAW. Sixteen alleles of *CUHas1* and *CUHas5* observed in at least one of the Gulf of Thailand samples was not found in HATRAW.

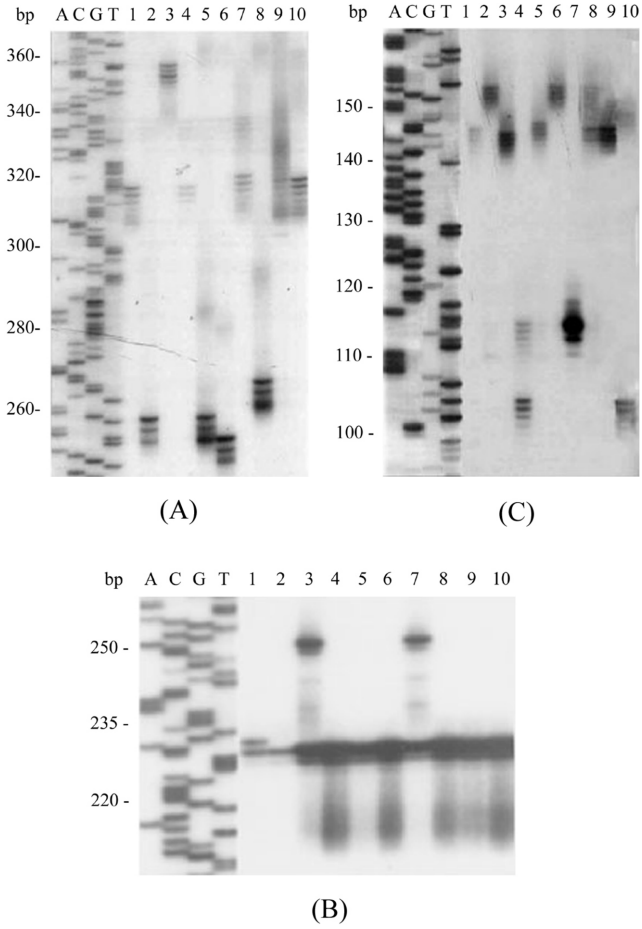

Fig. 4. Microsatellite patterns of non-related *H. asinina* (lanes 1-10) at *CUHas1* (A), *CUHas4* (B) and *CUHas5* (C) loci. A sequencing ladder of M13mp18 is included as the size standard.

Table 3. Allele frequencies, proportion of observed and expected heterozygosity of *CUHas1*, *CUHas4* and *CUHas5* loci across 4 conspecific samples of *H. asinina*

	Allele (bp)	HACAME	HACAMHE	HASAMHE	HATRAW
CUHas1	258	0.026	-	-	-
	260	0.053	-	-	-
	262	0.053	0.100	0.150	-
	266	0.026	-	-	-
	268	0.053	-	-	-
	272	0.026	0.033	0.050	-
	316	0.158	0.300	0.250	-
	318	-	0.033	-	-
	322	0.026	-	-	-
	324	0.026	-	-	-
	326	0.053	0.133	0.150	-
	328	0.132	0.067	0.050	-
	332	0.053	-	-	-
	334	0.053	0.067	0.100	0.018
	336	0.105	0.167	0.200	-
	338	0.026	-	-	-
	340	0.079	-	-	-
	344	0.026	-	-	0.036
	346	-	0.067	-	0.036
	348	-	-	-	0.089
	350	0.026	-	-	0.036
	352	-	-	-	0.161
	354	-	-	-	0.143
	356	-	-	-	0.339
	358	-	0.033	0.050	0.071
	360	-	-	-	0.071
Number of alleles		18	10	8	9
Number of effective alleles		12.21	6.16	6.06	5.45
H_o		0.84	0.87	0.80	0.86
H_e		0.92	0.84	0.84	0.82
CUHas4	222	-	0.600	-	0.450
	224	-	-	-	0.225
	228	0.864	-	0.550	0.325
	230	0.045	0.067	0.100	-
	250	0.091	0.333	0.350	-
Number of alleles		3	3	3	3
Number of effective alleles		1.32	2.10	2.30	2.79
H_o		0.27	0.40	0.50	0.50
H_e		0.24	0.52	0.57	0.64

Genotypic disequilibrium analysis indicated that genotypes of *CUHas1*, *CUHas4* and *CUHas5* were associated randomly ($p > 0.05$). The highest polymorphic loci across investigated samples was *CUHas1* ($H_o = 0.84$) followed by *CUHas4* ($H_o = 0.42$) and *CUHas5* ($H_o = 0.33$) with the number of alleles and effective alleles per locus of 26 (258-360 bp) and 13.93, 5 (222-250 bp) and 2.47 and 23 (104-163 bp) and 10.70 alleles, respectively.

Among natural abalone samples, HACAME (the average number of allele and observed heterozygosity per locus, H_o, = 11.33 alleles and 0.44, respectively) showed a greater number of allele per locus but a lower H_o than did HATRAW (6.33 alleles and $H_o = 0.61$). High genetic diversity levels were also observed in the hatchery stocks; HACAMHE (7.33 alleles and $H_o = 0.54$) and HASAMHE (6.00 alleles and $H_o = 0.53$).

All investigated samples conformed Hardy-Weinberg expectations at *CUHas1* and *CUHas4* ($p > 0.05$). Only HATRAW did not deviate from Hardy-Weinberg equilibrium at *CUHas5* after the sequential Bonferroni procedure was

Table 3. Continued

	Allele (bp)	HACAME	HACAMHE	HASAMHE	HATRAW
CUHas5	104	-	0.107	0.100	-
	108	-	-	-	0.071
	109	-	-	-	0.482
	110	-	-	-	0.304
	112	-	-	-	0.071
	113	-	-	-	0.018
	114	0.175	0.214	0.150	0.018
	115	0.050	-	-	-
	116	-	-	-	0.036
	117	-	0.036	-	-
	118	0.025	-	-	-
	119	0.025	-	-	-
	145	0.025	0.107	0.100	-
	146	-	0.179	0.250	-
	147	0.100	0.071	0.100	-
	149	0.125	0.036	0.050	-
	150	0.050	-	-	-
	151	0.100	-	-	-
	152	0.025	-	-	-
	154	0.150	0.179	0.250	-
	155	0.125	-	-	-
	162	0.025	-	-	-
	163	-	0.071	-	-
Number of alleles		13	9	7	7
Number of effective alleles		8.89	6.88	5.56	2.97
H_o		0.20*	0.36*	0.30*	0.46
H_e		0.89	0.85	0.82	0.66

Hardy-Weinberg equilibrium was tested for each sample across all loci.
*significant deviation from Hardy-Weinberg equilibrium (p<0.0001).

applied ($p > 0.0125$).

Geographic heterogeneity analysis and F_{ST} statistics was concordant and indicated significant differentiation between HATRAW and each of the Gulf of Thailand samples ($p < 0.0001$).

Discussion

Genetic diversity of *H. asinina* High levels of genetic diversity were found between different geographic samples of *H. asinina* based on RAPD and microsatellite analyses. This basic information is crucial for establishment of selective breeding and conservation programmes of *H. asinina* in Thai waters.

Levels of polymorphic RAPD bands (82.5%) of *H. asinina* was lower than that in *H. rubra* (93.33%; Huang *et al*., 2000) and mud crabs (*Scylla serrata*, *S. ocenaica* and *S. tranquabarica*, 98.91%; Klinbunga *et al*., 2000) but greater than that in the black tiger prawn (*Penaeus monodon*, 72.5%) (Tassanakajon *et al.* 1997) and oysters (*Crassostrea belcheri*, 53.23% and *C. iredalei* 77.67%; Klinbunga *et al.*, 2001) from different geographic locations in Thailand.

Several fixed RAPD fragments were observed in *H. asinina* (see also Klinbunga *et al*., 2004). We further tested species-specific nature of a 1700 bp band generated from UBC101 (N = 130) by genotyping of *H. ovina* (N = 95) and *H. varia* (N = 33) originating from different geographic locations in Thailand. Result indicated specificity of this RAPD marker to *H. asinina* suggesting that RAPD-PCR is a rapid and simple method for generating useful molecular markers in abalone.

More recently, Klinbunga *et al*., (2004) developed a sequence-characterised amplified region (SCAR) marker from a candidate *H. asinina*-specific RAPD fragment found in the present study (650 bp, UBC271). Species-specificity of the marker (312 bp) was found in *H. asinina* (N = 111) without false positive results in *H. ovina* (N = 73) and *H. varia* (N = 32).

RAPD is sensitive to reaction conditions (e.g. quality of the DNA template and $MgCl_2$ and primer concentrations). Moreover, the possibility of comigration of RAPD fragments having different sequences but similar sizes cannot be excluded. Accordingly, microsatellites were also included for

population genetic studies of *H. asinina*. Locus-specific primers designed from sequences of microsatellites containing clones of *H. asinina* would provide more valid and stable data for evaluation of genetic diversity levels and subsequent applications in breeding programmes of *H. asinina*.

Relatively high levels of genetic diversity were found in natural samples of *H. asinina* (HACAME and HASAME). Comparable levels of heterozygosity between hatchery stocks (HACAMHE and HSAMHE) and their natural samples suggested that founders contributed in the hatchery stocks were relatively large.

Selvamani *et al*. (2000) isolated and characterized 11 microsatellites in *H. asinina* but the genetic variability of these microsatellites was only examined in the Heron reef population, Australia (N = 21-41 per locus). The number of alleles per locus was 2-25 alleles and the expected heterozygosity (H_e) was 0.29-0.96. The level of genetic diversity of *H. asinina* in coastal Thai waters reported in this study was as high as that reported in *H. discus discus* (3-10 alleles, H_o = 0.18-0.80, Sekino and Hara, 2001) and *H. rubra* (8-41 alleles, H_o = 0.19-0.38; Huang *et al*., 2000 and 2-16 alleles, H_o = 0.14-0.76; Evans *et al*., 2000).

Intraspecific genetic differentiation of natural *H. asinina*

Large genetic distances from RAPD analysis and large numbers of non-overlapping alleles at *CuHas1* and *CuHas5* observed in natural samples originating from the Gulf of Thailand (HACAME and HASAME) and the Andaman Sea (HATRAW) suggested the existence of genetic subdivisions of Thai *H. asinina*.

Shephred and Brown (1993) predicted that microgeographic population differentiation within each abalone species should be occurred due to its short planktonic larval stages and limited dispersal ability. Accordingly, intraspecific genetic differentiation of abalone may be found within the scale of a few kilometers. In *H. asinina*, genetic differentiation was not observed between HACAME and HASAME located within the Gulf of Thailand (p = 0.2338-1.0000). Nevertheless, a neighbor-joining tree between pairs of samples, genetic heterogeneity analysis and *F*-statistics revealed significant genetic differentiation between those and HATRAW located in the Andaman Sea ($p < 0.0001$). This suggested that the gene pool of Thai *H. asinina* was not panmictic but reproductively isolated at the macrogeographic level to the Gulf of Thailand and the Andaman Sea populations.

Non-significant genetic heterogeneity between the hatchery stocks (HACAMHE and HASAME) and their corresponding natural samples (HACAME and HASAME) based on RAPD and microsatellite analyses illustrated the possibility to use these hatchery stocks to establish the genetic-based stock enhancement programmes at the local origins of *H. asinina*.

Using nuclear DNA markers like RAPD and microsatellite, clear genetic heterogeneity was observed between HATRAW and the Gulf of Thailand samples ($p < 0.0001$). In contrast, Klinbunga *et al*. (2003) determined genetic diversity and population differentiation of the same *H. asinina* samples using PCR-RFLP of 16S (mitochondrial) rDNA. Only one mitotype was found in all of the east coast samples (haplotype diversity = 0) reflected a lack of genetic heterogeneity in this species. Including 18S (nuclear) rDNA in the analysis resulted in an increase of haplotype diversity to 0.3391, 0.7816, 0.7511 and 0.7065 in HACAME, HACAMHE, HASAMHE and HATRAW, respectively. Under the presumption of selective neutrality of molecular markers and life history of *H. asinina*, female founder effects rather than biased female gene flow may have significantly affected contradictory population subdivision patterns (panmixia from mtDNA and reproductive isolation from nuclear DNA, respectively) when examined by different molecular markers.

Huang *et al.* (2000) examined genetic population structure of ten geographic samples of *H. rubra* in Australia using RAPD, minisatellites (GHR and MIPR) and microsatellites (RUBGT1, RUBCT1 and RUBGACA1). All types of DNA markers revealed significant genetic subdivisions of *H. rubra* along the coastline. Significant Hardy-Weinberg disequilibrium due to homozygote excess was observed at all microsatellite loci across all investigated samples. Apparently, deviation from Hardy-Weinberg expectations has been reported in other abalone genetically analysed by microsatellites including *H. kamtschatkana* (Miller *et al*., 2001), *H. discus discus* (Sekino and Hara, 2001) and *H. asinina* (this study). This may be caused from restricted larval recruitment patterns and asynchronous spawning over short geographic distances of abalone (Huang *et al*., 2000).

The genetic improvement of *H. asinina* cannot be achieved without knowledge on the control of growth, reproduction and defense mechanisms at the molecular levels. Genomic researches on gene discovery and development of marker-assisted selection (MAS) in *H. asinina* are useful but still in the initial stages. Amparyup *et al*. (2004) constructed normal and subtractive cDNA libraries from ovaries and testes of *H. asinina* and 588 randomly selected clones were unidirectional sequenced. Several functionally important transcripts related with biological and physiological processes of *H. asinina* were isolated. Expression of sex-related genes including homologues of axonemal p66.0, 18 kDa fertilization protein (FP), gonadotropin-inducible ovarian transcriptional factor1 (GIOT1), hydroxysteroid dehydrogenase (HSD), tektin A1, small androgen receptor interacting protein (SARIP), sperm lysin, vitelline coat protein (VCP) and vitellogenin subunit1 (VTG1) was further examined using RT-PCR. Gender-specific expression was found from homologues of VCP and VTG1 and axonemal p66.0, tektin A1 and sperm lysin in female and male *H. asinina*, respectively.

Besides knowledge from genome studies, the ability to identify population differentiation within a commercially important species like *H. asinina* is crucial for broodstock management and conservation programmes (Jarayabhand *et al*., 2002; Klinbunga *et al*., 2003). In the present study, we used RAPD and microsatellite analyses to elucidate the

existence of population differentiation of Thai *H. asinina* which was not able to be detected from the female-mediated marker like 16S rDNA polymorphism. The information suggested that *H. asinina* from the Gulf of Thailand and the Andaman Sea should be treated and managed separately. Practically, breeding programmes of *H. asinina* can be carried out more efficiently by incorporation of microsatellites. Offspring from multiple parents can be reared together allowing a better scrutiny of genetic effects on each economically important trait by reducing confounding environmental effects arisen when selected abalone families are cultured separately.

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