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# GENETIC VARIATION IN POPULATIONS OF WHITE SCAR (*CRASSOSTREA BELCHERI*) AND BLACK SCAR OYSTERS (*C. IREDALEI*) ALONG THE COAST OF THAILAND BY MEANS OF ISOZYMES

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ABSTRACT: A total of 229 individuals from eight populations of cultured and natural white scar oysters (*Crassostrea belcheri*), and a total of 255 individuals from ten populations of black scar oysters (*Crassostrea iredalei*), were sampled along the coast of Thailand and Malaysia. Analysis of nine enzymes using adductor muscle tissue gave a total of twelve loci in the white scar oyster and seven loci in the black scar oyster. No clear regional differentiation was found for the oysters, which might result from the large exchange of cultured oysters across Thailand for aquaculture. The lack of differentiation of the Malaysian sample was also thought to reflect the exchange of oysters for aquaculture between the Gulf of Thailand and Andaman Sea. One enzyme provided variation that was diagnostic for the two species.

## INTRODUCTION

Oysters are benthic marine species having a long planktonic larval stage (Avise, 1994). They live in near shore, shallow water bays and estuaries and are widely distributed throughout the world (Hedgecock, 1995).

Primarily on the basic of shell morphology, 15 oyster species belonging to seven genera from the families Ostreidae and Gryphaeidae are recognised in Thai waters (Klinbunga *et al.*, 2000). Two species within the genus *Crassostrea* (family Ostreidae, subfamily Crassostreinae) are found in Thailand, *i.e.* the white scar oyster, *Crassostrea belcheri* (Sowerby, 1871) and the black scar oyster, *C. iredalei* (Faustino, 1932). *C. iredalei* is a recent synonym for *C. lugubris* in Thailand (Yoosukh and Duangdee, 1999).

The large oysters *C. belcheri* and *C. iredalei*, and the smaller oyster *Saccostrea cucullata* have been cultured for at least fifty years in Thailand. *Crassostrea* oyster species are cultured in the Gulf of Thailand and the Andaman Sea by both intensive and extensive methods, using a range of techniques including bottom culture (intertidal and subtidal), stakes (cement pole, cement core), and racks (tray, net and string). Suspended culture is conducted on an experimental scale (Jarayabhand and Thavornyutikarn, 1995). Annual production between 1986 and 1990 was estimated to be approximately 1,500 tons (Department of Fisheries, 1993), and has been limited mainly by the lack of appropriate 'grow-out' techniques and by seasonal fluctuation in seed supply. Oyster seeds were collected entirely from nature, resulting in overexploitation of natural populations at different stages of development (Jarayabhand *et al.*, 1994).

Although oyster production from aquaculture has increased to approximately 20,000 tons annually since 1994, it still accounts for only 35 % of the total production (Department of Fisheries, 1998). Laboratory seed production of *S. cucullata* and large-scale hatcheries of *C. belcheri* have been successfully developed (Jarayabhand *et al.*, 1985; Sahavacharin *et al.*, 1988), providing the opportunity to develop selective breeding programs for increasing culture and management efficiency of Thai oysters.

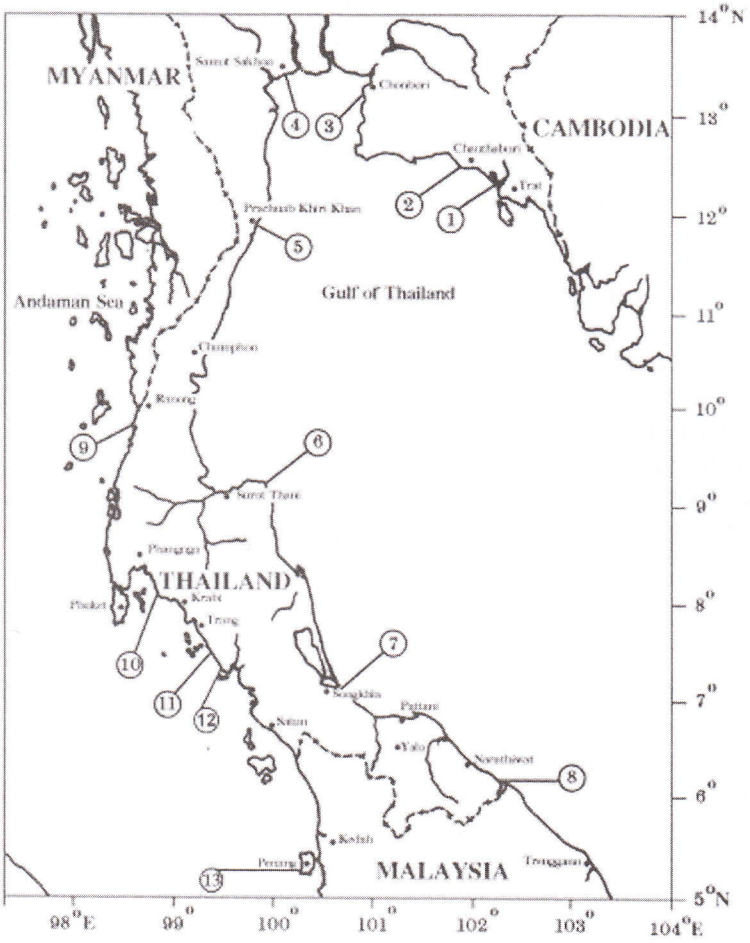


**Figure 1.** Sampling sites for white and black scar oysters in the Gulf of Thailand (1. Ban Salak, 2. Tha Mai, 3. Ang Sila, 4. Klong Pittayalongkorn, 5. Klong Bangnangrom, 6. Kanjanadit, 7. Nathap, 8. Klong Takbai), the Andaman Sea (9. Ao Khoa Yua, 10. Khok Krai, 11. Ratchamongkol, 12. Ban Tharua) and Malaysia (13. Penang).

Knowledge of genetic variation of commercial oysters in Thailand is important for establishing appropriate breeding programs and for management of natural populations of these species. The application of genetic markers would be helpful for selecting appropriate brood-stock for aquaculture production and for studies of larval distribution patterns and recruitment of Thai oysters. This information would enhance aquaculture output without adversely effecting native populations, leading to sustainable farming of these taxa (Hedgecock, 1995).

Although there are many reports on genetic variation using allozyme analysis in oysters from other parts of the world (Torigoe and Inaba, 1975a, b; Torigoe, 1978; Buroker *et al.*, 1979a, b; Buroker, 1984; Hedgecock and Okazaki, 1984; Ozaki and Fujio, 1985; English *et al.*, 2000), the information on tropical commercial *Crassostrea* oysters, especially in Thailand, is quite limited. Poltanya (1985) observed differences between white scar and black scar in Thailand using allozymes. Comparison of populations of black scar oysters from the Gulf of Thailand, the eastern

**Table 1.** List of sampling sites for white and black scar oysters in Thailand and Malaysia, 2000–2001.

| Region | Province or country | Locality | Species | Stock |
|---|---|---|---|---|
| Gulf of Thailand | Trat | 1. Ban Salak | Black scar | Aquaculture |
| | | | White scar | Aquaculture |
| | Chanthaburi | 2. Tha Mai | White scar | Natural |
| | Chon Buri | 3. Ang Sila | Black scar | Aquaculture |
| | Samut Sakhon | 4. Klong Pittayalongkorn | Black scar | Aquaculture |
| | Prachuab KhiriKhan | 5. Klong Bangnangrom | Black scar | Aquaculture and natural at pier |
| | Surat Thani | 6. Kanjanadit | White scar | Aquaculture |
| | Songkla | 7. Nathap | Black scar | Natural |
| | Narathiwat | 8. Klong Takbai | Black scar | Aquaculture |
| | | | White scar | Aquaculture |
| Andaman Sea | Ranong | 9. Ao Khoa Yua | Black scar | Natural |
| | | | White scar | Natural |
| | Phangnga | 10. Khok Krai | Black scar | Aquaculture |
| | | | White scar | Aquaculture |
| | Trang | 11. Ratchamongkol | White scar | Aquaculture |
| | | 12. Ban Tharua | White scar | Natural |
| | Malaysia | 13. Penang | Black scar | Aquaculture |

coast of Malaysia and eastern coast of south India indicated the Indian population differed from the Malaysian and Thai populations (Murugan *et al.*, 1999).

Species-specific markers were found *C. belcheri, C. iredalei* and *Saccostrea cucullata*, but not in *S. forskali* and *Striostrea (Parastriostrea) mytiloides* by random amplified polymorphic DNA (RAPD) analysis using material from Thailand (Klinbunga *et al.*, 2000). Development of species-specific markers of each oyster species would allow direct examination of the correct larvae and broodstock species and avoid the use of a part of the species complexes for aquaculture (Banks *et al.*, 1993; Littlewood, 1994).

The aim of this study is to compare genetic variation in populations of white scar and black scar oysters along the coast of Thailand by means of isozymes and to establish the population genetic structure of these species in Thailand to assist future selective breeding in aquaculture work.

## MATERIALS AND METHODS

### 1. Sampling sites and transport and storage

White scar and black scar oysters were sampled during 2000–2001 from natural and aquaculture populations along the coast of Thailand and Malaysia. Eight locations were situated in the Gulf of Thailand and five in the Andaman Sea including one sample from Penang, Malaysia (see Table 1 and Fig.1). The adductor muscles of collected oysters were dissected out and stored at –80 °C until analysis.

### 2. Isozyme analysis

The adductor muscle was thawed and homogenised with an approximately equal amount of 1% polyvinylpyrrolidone in a tris-citrate buffer pH = 7.0 and centrifuged at 5,000 rpm for 10 minutes. The supernatant was applied to a 12% starch gel (Connaught starch) by soaking the supernatant into 9 x 4 mm paper wicks (Whatman

**Table 2.** Enzymes tested in two *Crassostrea* species (*C. belcheri* and *C. iredalei*), the abbreviation of the enzyme, E.C. number, buffer used and number of loci expressed in each species.

| Enzyme | Abbre-viation | E.C. Number | Buffer used | Number of loci *C. belcheri* | *C. iredalei* |
|---|---|---|---|---|---|
| Aspartate aminotransferase | AAT | 2.6.1.1 | A | 1 | 1 |
| Cytosol aminopeptidase | AP | 3.4.11.1 | A | 1 | 1 |
| Glucosephosphate isomerase | GPI | 5.3.1.9 | B | 1 | 1 |
| Isocitrate dehydrogenase | IDH | 1.1.1.42 | B | 2 | 2 |
| Malate dehydrogenase | MDH | 1.1.1.37 | B | 2 | 2 |
| Mannose-6-phosphate isomerase | MPI | 5.3.1.8 | B | 1 | 1 |
| Peptidase (leucyl-glycyl-glycine) | PEP(LGG) | 3.4.11.13 | B | 2 | 1 |
| Peptidase (leucyl-tyrosine) | PEP(LT) | 3.4.11.13 | A | 1 | 1 |
| Phosphoglucomutase | PGM | 5.4.2.2 | B | 1 | 1 |

3 MM chromatography paper) and inserted them in the gel. The nine enzymes were tested on two buffers (Table 2).

Performance of electrophoresis and staining procedures were according to Selander *et al.* (1971) and Richardson *et al.* (1986).

Enzyme abbreviations are written with capital letters, *e.g.* AAT (Table 2), and the corresponding locus is written in italics, *e.g. AAT*. Loci were numbered according to their migration rate towards the anode, *e.g. AAT-1*, *AAT-2* and alleles were named 1, 2, 3 *etc.* according to their migration rate towards the anode.

### 3. Data analysis

The software G-STAT (Siegismund, 1995) was used for data analysis regarding tests for fit to Hardy-Weinberg expectations, for estimation of sample statistics [the fraction of polymorphic loci at 99% level ($P_{99}$, *i.e.* the frequency of the most common allele did not exceed 99%), the number of alleles (A), the observed ($H_o$) and expected ($H_e$) heterozygosity] and for estimating the genetic identity according to Nei (1972). Bonferroni corrections were used for multiple simultaneous analyses (Rice, 1989). Genetic Data Analysis (GDA) software (Lewis and Zaykin, 2001) was used for the estimation of genetic distance for construction of dendrograms using the UPGMA algorithm. The software TREEVIEW (Page, 1996) was used for depicting the dendrogram. F-stat (Goudet, 2001) was used for pairwise tests of differentiation.

## RESULTS

Nine enzymes were tested in both species representing twelve loci in *C. belcheri* and eleven loci in *C. iredalei*. However, AP, MPI, PEP(LGG) and PEP(LT) were difficult to score in black scar oysters and were omitted from the data analyses, so only 7 loci were considered here.

### 1. White scar *C. belcheri*

Thirty seven out of 51 tests for fit to Hardy-Weinberg expectations revealed an excess of heterozygotes, but only one showed a significant deficit of heterozygotes after Bonferroni correction. The ratio of excess and deficiency of heterozygotes, 37:14, deviated significantly ($\chi^2 = 10.37$, d.f. = 1, $P < 0.001$) from the expected 1:1 ratio.

The proportion of polymorphic loci ranged between 0.50–0.75, the average number of alleles (A) between 1.67–2.17 and the observed ($H_o$) and expected ($H_e$) heterozygosity both between 0.08 –0.17 (Table 3).

The genetic identity between population pairs was high (0.95–0.98), but a number of the pairs showed significant differences in allelic frequencies

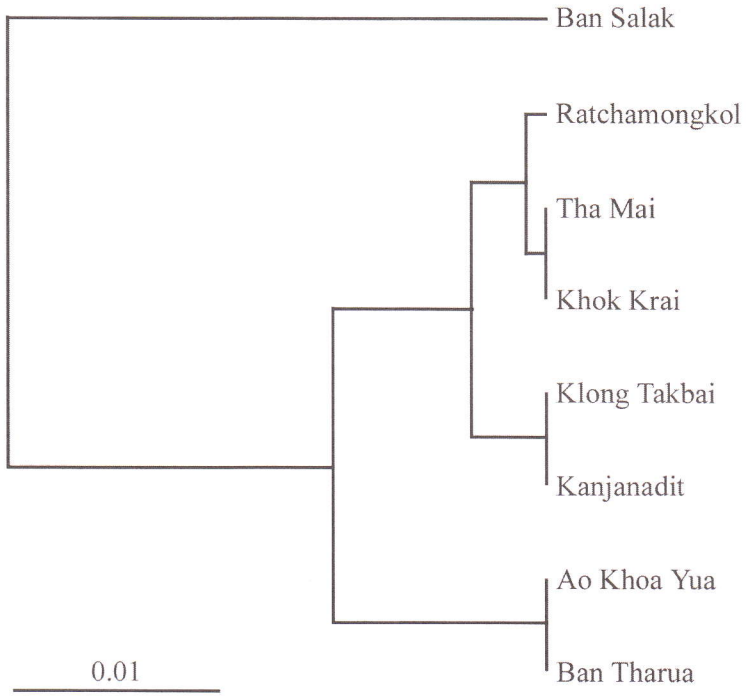


**Figure 2**. Dendrogram showing the relationships between populations of white scar oyster, based on 10 loci.

**Table 3**. List of number of loci analysed (n), the averages of number (N) of individuals of white scar oysters analysed, $P_{99}$, A, $H_o$ and $H_e$ ($P_{99}$ is the fraction of loci where the frequency of the most common allele does not exceed 99%, A the actual number of alleles, $H_o$ the observed heterozygosity and $H_e$ the expected heterozygosity). The averages are given with the standard deviations.

| Region | Locality | n | N | $P_{99}$ | A | $H_o$ | $H_e$ |
|---|---|---|---|---|---|---|---|
| Gulf of Thailand | Ban Salak | 12 | 26.3±4.8 | 0.50 | 1.92±1.00 | 0.08±0.11 | 0.08±0.12 |
| | Tha Mai | 12 | 18.7±3.1 | 0.50 | 1.67±0.89 | 0.08±0.09 | 0.09±0.11 |
| | Kanjanadit | 10 | 27.9±6.6 | 0.60 | 1.80±0.92 | 0.14±0.21 | 0.15±0.22 |
| | Klong Takbai | 10 | 29.3±1.2 | 0.60 | 2.00±0.94 | 0.14±0.19 | 0.14±0.16 |
| Andaman Sea | Ao KhoaYua | 12 | 29.3±0.9 | 0.58 | 2.00±0.95 | 0.13±0.13 | 0.14±0.14 |
| | Khok Krai | 12 | 27.6±4.1 | 0.50 | 1.83±0.94 | 0.10±0.13 | 0.11±0.14 |
| | Ratchamongkol | 12 | 25.4±5.1 | 0.75 | 2.17±0.94 | 0.13±0.15 | 0.13±0.14 |
| | Ban Tharua | 12 | 27.7±4.9 | 0.50 | 1.83±0.94 | 0.17±0.20 | 0.17±0.18 |

although there was no obvious geographical pattern to these (Table 4). Since two samples were not scored for AP and MPI, these loci were omitted when estimating genetic distances. The distances were used to construct a dendrogram, which revealed no clear distinction between the Gulf of Thailand and the Andaman Sea (Fig. 2).

**2. Black scar, *C. iredalei***

Two of the 40 tests for deviation from Hardy-Weinberg expectations deviated significantly after Bonferroni corrections, both were found in the sample from Malaysia. The distribution of samples with excess and deficiency of heterozygotes did not differ statistically from the expected 1:1 ratio, *i.e.* 25:15 ($\chi^2 = 2.50$, d.f. = 1, $P < 0.11$).

**Table 4.** Genetic identity above the diagonal and tests for differentiation between populations below the diagonal for white scar from Thailand.

| Locations | Ban Salak | Tha Mai | Kanjanadit | Klong Takbai | Ao Khao Yua | Khok Krai | Ratchamongkol | Ban Tharua |
|---|---|---|---|---|---|---|---|---|
| Ban Salak | - | 0.98 | 0.95 | 0.97 | 0.98 | 0.97 | 0.97 | 0.97 |
| Tha Mai | n.s. | - | 0.96 | 0.98 | 0.98 | 0.98 | 0.97 | 0.97 |
| Kanjanadit | * | n.s. | - | 0.97 | 0.96 | 0.98 | 0.97 | 0.96 |
| Klong Takbai | * | n.s. | n.s. | - | 0.98 | 0.98 | 0.98 | 0.98 |
| Ao Khao Yua | n.s. | n.s. | * | n.s. | - | 0.98 | 0.97 | 0.98 |
| Khok Krai | n.s. | n.s. | n.s. | n.s. | n.s. | - | 0.98 | 0.97 |
| Ratchamongkol | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | - | 0.97 |
| Ban Tharua | * | n.s. | * | n.s. | n.s. | n.s. | n.s. | - |

n.s. = not significant
* significant after Bonferroni corrections

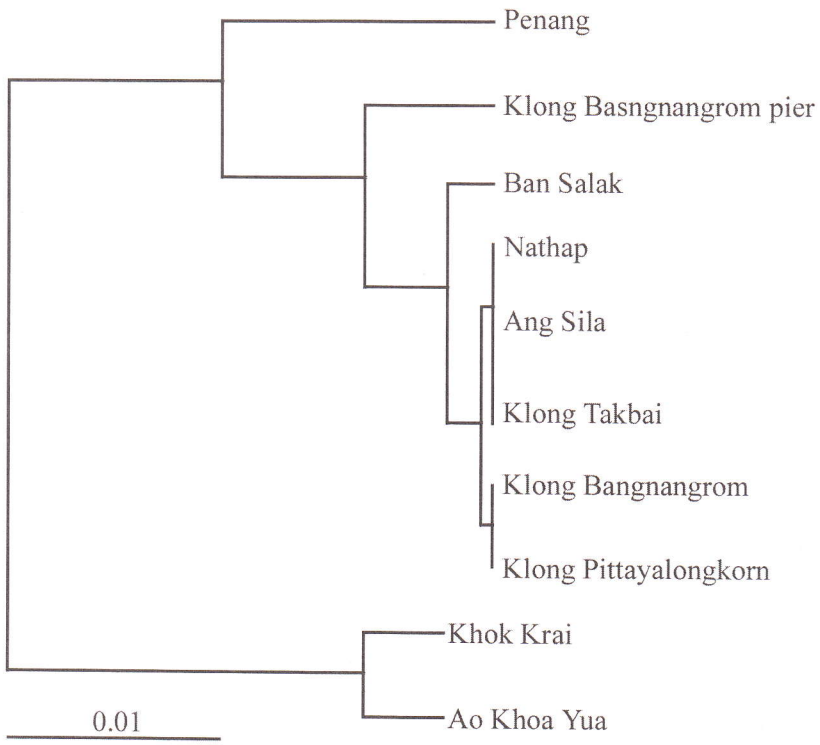


**Figure 3**. Dendrogram showing the relationships between populations of black scar oyster, based on 7 loci.

The proportion of polymorphic loci ranged between 0.29–0.71, the average number of alleles (A) between 1.86–3.14 and the observed heterozygosity ($H_o$) between 0.18–0.26 and the expected ($H_e$) heterozygosity between 0.19–0.29 (Table 5).

The genetic identity between population pairs ranged from 0.92 to 0.98, but several pairwise tests for differentiation revealed significant deviation. However, there was no obvious pattern in the deviations. A dendrogram based on analysis of genetic distance showed two main groups, one consisting of the two samples from the Andaman Sea coast of Thailand, and the other consisting of samples from the Gulf of Thailand and Malaysia (Fig. 3).

**Table 5.** List of number of loci analysed (n), the averages of number (N) of individuals of black scar oysters analysed, $P_{99}$, A, $H_o$ and $H_e$ ($P_{99}$ is the fraction of loci where the frequency of the most common allele does not exceed 99%, A the actual number of alleles, $H_o$ the observed heterozygosity and $H_e$ the expected heterozygosity). The averages are given with the standard deviations.

| Region | Locality | n | N | $P_{99}$ | A | $H_o$ | $H_e$ |
|---|---|---|---|---|---|---|---|
| Gulf of Thailand | Ban Salak | 7 | 28.0±4.8 | 0.43 | 2.00±1.41 | 0.21±0.34 | 0.20±0.31 |
| | Ang Sila | 7 | 28.6±3.8 | 0.57 | 2.57±1.62 | 0.21±0.32 | 0.23±0.33 |
| | Klong Pittayalongkorn | 7 | 22.0±0.0 | 0.57 | 2.29±1.60 | 0.18±0.30 | 0.21±0.33 |
| | Klong Bangnangrom[a] | 7 | 37.1±3.3 | 0.43 | 2.43±2.15 | 0.22±0.36 | 0.21±0.35 |
| | Klong Bangnangrom[b] | 7 | 19.1±2.3 | 0.71 | 2.43±1.51 | 0.20±0.27 | 0.20±0.29 |
| | Nathap | 7 | 9.00±0.0 | 0.29 | 1.86±1.46 | 0.22±0.39 | 0.19±0.32 |
| | Klong Takbai | 7 | 29.9±0.4 | 0.71 | 2.71±1.80 | 0.22±0.31 | 0.24±0.34 |
| Andaman Sea | Ao Khoa Yua | 7 | 21.1±1.9 | 0.85 | 3.14±2.04 | 0.21±0.27 | 0.26±0.36 |
| | Khok Krai | 7 | 29.0±3.6 | 0.71 | 2.86±1.95 | 0.26±0.33 | 0.29±0.35 |
| | Penang | 7 | 17.9±4.7 | 0.43 | 2.00±1.53 | 0.15±0.26 | 0.23±0.33 |

[a] aquaculture population
[b] natural population

**Table 6.** Genetic identity above the diagonal and tests for homogeneity below the diagonal for black scar from Thailand and Malaysia.

| Locations | Ban Salak | Ang Sila | Klong Pittaya longkorn | Klong Bangnang rom[a] | Klong Bangnang rom[b] | Nathap | Klong Takbai | Ao KhoaYua | Khok Krai | Penang |
|---|---|---|---|---|---|---|---|---|---|---|
| Ban Salak | - | 0.97 | 0.98 | 0.98 | 0.97 | 0.96 | 0.98 | 0.94 | 0.96 | 0.96 |
| Ang Sila | n.s. | - | 0.97 | 0.97 | 0.96 | 0.95 | 0.97 | 0.95 | 0.97 | 0.96 |
| Klong Pittayalongkorn | n.s. | n.s. | - | 0.98 | 0.96 | 0.95 | 0.97 | 0.94 | 0.96 | 0.96 |
| Klong Bangnangrom[a] | n.s. | n.s. | n.s. | - | 0.96 | 0.96 | 0.98 | 0.95 | 0.97 | 0.97 |
| Klong Bangnangrom[b] | n.s. | n.s. | n.s. | n.s. | - | 0.94 | 0.96 | 0.93 | 0.95 | 0.94 |
| Nathap | n.s. | n.s. | n.s. | n.s. | n.s. | - | 0.96 | 0.92 | 0.94 | 0.94 |
| Klong Takbai | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | - | 0.96 | 0.97 | 0.97 |
| Ao Khoa Yua | * | n.s. | n.s. | * | n.s. | n.s. | n.s. | - | 0.96 | 0.95 |
| Khok Krai | * | n.s. | * | * | n.s. | n.s. | * | n.s. | - | 0.96 |
| Penang | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | * | - |

[a] aquaculture population
[b] natural population
n.s. = not significant

* significant after Bonferroni corrections

**3. Diagnostic loci for white scar and black scar oyster**

The two species showed fixed allele difference at only *MDH-2* in which the white scar oysters possessed a fast migrating allele compared to black scar. Obvious differences in the allelic frequencies were found at *GPI*, *IDH-2* and *PGM* but not at *AAT*, *IDH-1* and *MDH-1*.

## DISCUSSION

**1. White scar oysters**

The only sample, which revealed a significant deficit of heterozygotes was from Kanjanadit, Surat Thani at *GPI*. In 1996 the populations at Surat Thani were exposed to a massive die-off (approximately 90%) (Klinbunga *et al.*, 2001). A stock enhancement program was initiated and if the source populations used for the enhancement had different allelic frequencies, the Wahlund effect may explain the deficiency of heterozygotes. However, none of the other polymorphic loci revealed a severe deficiency of heterozygotes. This might be due to a lack of differentiation among the other loci. The observation might be biased also by the low sample size (nine individuals) for that population. The presence of null alleles might be another explanation for the observed deficiency of heterozygotes (*e.g.* Simonsen and Kittiwattanawong, 1998).

The levels of heterozygosity observed in this study was higher than that observed by Buroker *et al.* (1979b) who collected samples of natural populations of *C. belcheri* living at Tawau, Sabah, North Borneo, Eastern Malaysia. These authors analysed 25 loci compared to 12 in this study, and the array of loci was not the same. This might cause the difference in the level of heterozygosity observed. Klinbunga *et al.* (2001) reported a high heterozygosity when random amplified polymorphic DNA analysis (RAPD-PCR) was applied for studying the Thai oyster genera *Crassostrea, Saccostrea* and *Striostrea.* In addition, a possible subdivision of populations was also observed in *C. belcheri*.

The similarity between Ao Khoa Yua and Ban Tharua on the West Coast of Thailand and between Kanjanadit and Klong Takbai and in the Gulf might be a result of geographical separation, *i.e.* the Andaman region and the Gulf of Thailand region. However, the similarity among Khok Krai, Ratchamongkol and Tha Mai, representing locations both on the West Coast and the East Coast, could be explained by transplantation practices by farmers for their brood-stock enhancement and commercial purposes (whiter meat colour). Transplantation of brood-stocks is known to take place across Thailand. The sample from Ban Salak in the Eastern part of the Gulf seems to be to distinct from the other samples, but nothing in the history of this culturing site could explain divergence of this sample. However, it has to be emphasized that the differentiation was low as shown in Table 4.

**2. Black scar oysters**

*GPI* and *IDH-1* in the sample from Penang, Malaysia both showed a significant lack of heterozygotes. This might be due to the sample size or mixing of two or more populations as the sample was bought from local dealers who might have mixed populations.

This present study revealed higher heterozygosity in *C. iredalei* than reported by Buroker *et al.* (1979b), who collected from natural habitats at Binakayan, Cavite, Philippines. However, the number of loci in the present study was less than the number studied by Buroker *et al.* (1979b). High level of genetic variation was also reported when applying the RAPD-PCR analysis technique to the Thai black scar oyster (Klinbunga *et al.*, 2001).

The dendrogram relating to black scar oysters revealed two major groups, one representing the Thai samples from the Andaman Sea, and the other represents the samples from the Gulf of Thailand and, surprisingly, the sample from Penang, Malaysia. An explanation for the closer relationship between the Malaysian sample and the samples from the Gulf of Thailand could be that most of the samples sold in Penang actually come from the eastern coast of Malaysia (such as Trengganu) adjacent to the Gulf of Thailand. Furthermore, trade dealers in Penang sometimes import / black scars from Thailand through the Had Yai border, Songkla province. Black scars are also considered as high

value food in Malaysia apart from white scars (Tan Shau-Hwai, pers. comm.), whereas white scars are considered as high value food in Thailand.

### 3. Diagnostic loci for the two species white scar and black scar oyster

Buroker *et al.* (1979b) found thirteen diagnostic loci for *C. belcheri* and *C. iredalei* among twenty-three loci investigated in both species. They studied a sample of *C. belcheri* from Tawau, Sabah, North Borneo, Malaysia and a sample of *C. iredalei* from Binakayan, Cavite, Philippines. Comparison of this study with the study of Buroker *et al.* (1979b) was difficult, because the authors did not mention if the loci were numbered according to migration rate or the distance from the application line. However, both studies found that the enzyme MDH was useful for differentiating the two species.

## CONCLUSION

The fact that the majority of the samples for this study were obtained from aquaculture populations means that we cannot exclude the possibility that a number of aspects of population genetic diversity, including some differences between populations, may result from translocations or hatchery processes. However, it seems reasonable that some regional differentiation occurs in black scar oysters but that the transportation of seeds of white scar oysters has obscured any regional differences that may have been present in that species.

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## REFERENCES

Avise, J.C. 1994. Molecular markers, natural history and evolution. London: Chapman and Hall. 511 p.

Banks, M.A., D. Hedgecock and C. Waters. 1993. Discrimination between closely related Pacific oyster species (*Crassostrea*) via mitochondrial DNA sequences coding for large subunit rRNA. Mol. Mar. Biol. Biotechnol. **2**: 129–136.

Buroker, N.E. 1984. Gene flow in the mainland and insular population of *Crassostrea* (Mollusca). Biol. Bull. **166**: 550–557.

Buroker, N.E., W.K. Hershberger and K.K. Chew. 1979a. Population genetics of the family Ostreidae: I. Intraspecific studies of *Crassotrea gigas* and *Saccostrea commercialis*. Mar. Biol. **54**: 157–169.

Buroker, N.E., W.K. Hershberger, and K.K. Chew. 1979b. Population genetics of the family Ostreidae: II. Interspecific studies of the genera *Crassostrea* and *Saccostrea*. Mar. Biol. **54**: 171–184.

Department of Fisheries. 1993. Fisheries statistics of Thailand 1991. Department of Fisheries, Ministry of Agriculture and Cooperatives, no. 3/1989, 94 p.

Department of Fisheries 1998. Fisheries Statistics of Thailand 1995. Department of Fisheries, Ministry of Agriculture and Cooperatives, no. 5/1998, 86 p.

English, L.J., G.B. Maguire and R.D.Ward. 2000. Genetic variation of wild and hatchery populations of the Pacific oyster, *Crassostrea gigas* (Thunberg), I. Australia. Aquaculture **187**: 283–298.

Faustino, L.A. 1932. Recent and fossil shells from the Philippine Island I. Phillip. J. Sci. **49**(4): 543–549.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat. html.

Hedgecock, D. 1995. The cupped oyster and the Pacific oyster. **In**: Thrope, J., G.G. Gall, J. Lannan, and C. Nash (eds.). Conservation of fish and shellfish resources: managing diversity American Press, London, pp.115–137.

Hedgecock, D. and N.B. Okazaki. 1984. Genetic diversity within and between populations of American oysters (*Crassostrea*). Malacologia **25**(2): 535–549.

Jarayabhand, P., S. Jaraeontia, C. Srisaard, and P. Menasveta. 1994. Experiments on larviculture of Thai oyster species. Thailand. J. Aquat. Sci. **1**(1): 43–53.

Jarayabhand, P., S. Rungsupa, N. Chaithanavisuti and P. Kanjanamawin. 1985. A preliminary study on the induced spawning of oyster (*Crassostrea* spp.) in Thailand species. Proceeding of Living Aquatic Resources, Chulalongkorn University, pp. 272–285 (in Thai with English abstract).

Jarayabhand, P. and M. Thavornyutikarn. 1995. Realized heritability estimation on growth rate of oyster, *Saccostrea commercialis* Born, 1778. Aquaculture **138**: 111–118.

Klinbunga, S., P. Ampayup, A. Tassanakajon, P. Jarayabhand and W. Yoosukh. 2000. Development of species-specific markers of the tropical oyster (*Crassostrea belcheri*) in Thailand. Mar. Biotechnol. **2**: 476–484.

Klinbunga, S., P. Ampayup, A. Tassanakajon, P. Jarayabhand and W. Yoosukh. 2001. Genetic diversity and molecular markers of cupped oyster (Genera *Crassostrea, Saccostrea* and *Striostrea*) in Thailand revealed by Randomly Amplified Polymorphic DNA Analysis. Mar. Biotechnol. **3**: 133–144.

Lewis, P.O. and D. Zaykin. 2001. Genetic data analysis: Computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/soft-ware.html

Littlewood, D. T. J. 1994. Molecular phylogenetic of cupped pysters based on partial 28S rDNA gene sequences. Mol. Phylogenet Evol. **3**:221–239.

Murugan A., M. Niklasson, S. Bussarawit, C. Aungtonya and F. Boneka. 1999. Allozyme comparison of black scar oyster populations of India, Thailand and Malaysia. Phuket mar. biol. Cent. Spec. Publ. **19**: 139–144.

Nei, M. 1972. Genetic distance between populations. Am. Nat. **106**: 283–292.

Ozaki, H. and Y. Fujio. 1985. Genetic differentiation in geographical populations of the Pacific oyster (*Crassostrea gigas*) around Japan. Tokyo J. Agri. Res. **36**(1): 49–61.

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences **12**: 357–358.

Poltanya, R. 1985. The isozyme studies on *Crassostrea belcheri* and *Crassostrea lugubris*. Proceeding of the Department of Fisheries Seminar, Bangkok. pp. 249–252.

Richardson, B.J., P.R. Baverstock and A. Adams. 1986. Allozyme electrophoresis: A handbook for animal systematics and population studies. Academic Press, Inc. San Diego, California. 410 p.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution **43**: 223–225.

Sahavacharin, S., A. Chindanon, S. Amornjaruchit, J. Nugranad, K. Silapajarn, V. Chawivanskorn, S. Limsurat, C.L. Angell, E.W. McCoy, K. Maturasint and M. Potaros. 1988. Hatchery techniques for tropical bivalve molluscs. **In**: McCoy, E.W. and T. Chongpepien (eds.). Bivalve Mollusc Culture Research in Thailand. ICLARM Technical Report **19**, pp. 19–30.

Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson and J.B. Gentry. 1971. Biochemical polymorphism and systematics in the genus Peromyscus variation in the old field mouse (*Peromyscus polionotus*). Studies of Genetics VI, University of Texas Publication **1703**: 49–90.

Siegismund, H.R. 1995. G-stat, version 3.1, genetical statistical programs for the analysis of population data. Department of Plant Ecology, Botanical Institute, Oester Farimagsgade 2d, DK-1353 Copenhagen K, Denmark.

Simonsen V., and K. Kittiwattanawong. 1998. Marine molluscs and molecular markers: Protein electrophoresis. Phuket mar. biol. Cent. Spec. Publ. **18**: 223–236.

Sowerby, G.B. Jr., 1871. Monograph of the genus *Ostraea*. **In**: L.A. Reeve's Conchologia Iconica. L. Reeve and Co., London, Vol. 18, 33 pls.

Torigoe, K. 1978. Electrophoretic variants of adductor muscle proteins in *Crassostrea gigas*. Jap. J. Malac. (Venus) **37**(4): 241–244.

Torigoe, K. and A. Inaba. 1975a. A comparison between *Ostrea denselamellosa* and *Ostrea futamensis* using electrophoresis on muscle proteins. Jap. J. Malac. (Venus) **34**: 93–98.

Torigoe, K. and A. Inaba. 1975b. Electrophoretic studies on some oysters. Jap. J. Malac. (Venus) **33**: 177–183.

Yoosukh, W. and T. Duangdee. 1999. Living oysters in Thailand. Phuket mar. biol. Cent. Spec. Publ. **19**: 363–370.

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