CHAPTER IV

DISCUSSION

Population genetic studies in abalone provided important information for both breeding and conservation in these taxa. Nevertheless, there have been no reports on this and related topics in Thai abalone (*H. asinina*, *H. ovina* and *H. varia*). One of the most difficult problems for genetic studies of abalone is collection of appropriate specimens to be studied by effective techniques. The sample collection sites in this study were essentially different from data previously reported by Geiger (2000) who did not report that *H. asinina* is existent in the Gulf of Thailand and *H. varia* is commonly found in 317 points of the world. Practically, *H. asinina* is available in the Gulf of Thailand but it was not possible to collect *H. asinina*. The latter species was only found as a rare species in the west of peninsular Thailand (Andaman Sea).

The reason to explain contradiction between data of Geiger (2000) and those of the present study is that most publication of species and distribution of abalone in Thai waters are in Thai whereas database from Geiger (2000) were accumulated from the past finding published in English. Among three Thai abalone, *H. varia* has been regarded as the most abundant species in the west of peninsular Thailand. It could commonly be found in Phuket and neighboring provinces. Overexploitation and destruction of the habitats are probably resulted in decreasing of *H. varia* rapidly.

DNA extracted from hemolymp, live and frozen specimens and those preserved in absolute ethanol provided comparable DNA quality to be used for RAPD analysis if DNA extraction was carried out as soon as possible. Specimens died during the collection period provided severe degraded genomic DNA suggesting a low quality for RAPD analysis.

Molecular markers are powerful tools for analysis of genetic relationships and diversity at different taxonomic levels. Hadrys *et al.*, (1992) illustrated advantages of RAPD analysis in various applications in molecular genetics and ecology including its suitability to examine genome which is not well studied using only limited quantity of DNA in a rapid time period. As a result, RAPD is one of the potential techniques for genetic investigation of various organisms particularly when dealing with a large number of specimens.

In the present study, five primers (OPB11, UBC101, UBC195, UBC197 and UBC271) were selected for RAPD analysis. However, the last three primers provided difficulties for amplification of *H. ovina* and *H. varia* DNA. They were then used for determination of intraspecific genetic diversity of *H. asinina* while OPB11 and UBC101 were used for analysis of diversity in three abalone species. Notably, the annealing temperature for RAPD-PCR of the primer UBC271 was 40° C (high temperature amplification, HAT-RAPD) which gave more reproducible patterns than the typical annealing temperature at 36° C (low temperature amplification, LAT-RAPD)

RAPD analysis revealed high genetic diversity of abalone in Thailand. The percentage of polymorphic bands in *H. asinina*, *H. ovina* and *H. varia* was 84.91%, 94.74% and 91.23%, respectively. The polymorphic band levels in abalone is greater than 47.92% - 77.59% in mud crabs (*Scylla serrata, S. oceanica* and *S. transquebarica*) from

eastern Thailand (Klinbunga *et al.*, 2000), 24.2%-47.8% in the black tiger shrimp (*P. monodon*) from different geographic locations in Thailand (Tassanakajon *et al.*, 1997) and 53.23%-77.67% of Crassostrea oysters but as high as Saccostrea and Striostrea oysters in Thailand (Klinbunga *et al.*, 2001).

Nevertheless, the percentage of polymorphic bands in RAPD analysis is largely dependent on taxonomic levels and geographic scales of taxa under investigation therefore this parameter should be considered with similarity indices and genetic distances.

Using primers OPB11 and UBC101, the similarity index within geographic samples indicated closer genetic relations between *H. asinina* sample (0.7927-0.8496) than *H. ovina* (0.6010-0.7032) and *H. varia* (0.5259-0.6102) samples. Genetic distances of abalone at the interspecific level (0.1578-0.4827) were greater than those at intraspecific levels (0.4127-0.7550). These differences were as similar as genetic distances in mud crabs (0.425-0.751; Klinbunga et al., 2000) but much less than those in the freshwater bryozoan, *Cristatella muceda* (0.9633-0.9977; Okamura *et al.*, 1993).

A lower level of genetic differences within *H. asinina* than *H. ovina* and *H. varia* can be explained by the potential mobility of *H. asinina* than other two species. This may have homogenized differentiation between geographically different samples of *H. asinina*. In contrast, genetic differentiation between the Gulf of Thailand and west of peninsular Thailand samples of *H. ovina* is observed owing to lower genetic differences within regions than between regions.

Although high genetic diversity of *H. varia* was found, results from this species should be considered with caution as the number of samples and sample sizes are limited (only 2 sample sites with N = 28 and 4 for specimens from Phuket and Phangnga,

respectively). Nevertheless, the percent of polymorphic bands, similarity index and genetic distance within a Phuket sample was higher than any sample of *H. asinina* suggesting the high genetic diversity in this rare species.

Shepherd and Brown (1993) predicted that population differentiation within each abalone species should be occurred due to short planktonic larval stages and their limited dispersal ranges. Genetically differentiated populations may be found within the scale of a few kilometers. Nevertheless, results from this study revealed clear genetic diversity within *H. ovina* from the west (Andaman) and the east (Gulf) coasts of peninsular Thailand, partial differentiation between *H. asinina* from the Gulf of Thailand and the Andaman and the Philippines but lack of differentiation within the same coastal regions in *H. asinina* and *H. varia* and possibly in *H. varia*. Therefore, factors relevant with population differentiation is not simply explained by the period of planktonic larval stages and dispersing ability of adults and should be further examined in details.

To consider genetic diversity within *H. asinina*, the average parameters from 5 primers would provide more accurate data than data from only OPB11 and UBC101. The average genetic distance of *H. asinina* within the Gulf of Thailand was 0.0243 (0.0156-0.0317) whereas the distance between each of the Gulf samples and *H. asinina* from Talibong and Philippines were 0.2096-0.2381. Results suggested partial differentiation between *H. asinina* from the Gulf of Thailand and the further east sample (Philippines) and the different coastal sample (Talibong).

The genetic diversity of three hatchery stocks (P_0 for HASH and HACH and F_1 for HAPH) exhibited similar levels of genetic diversity as that of natural *H. asinina*. This may

be resulted from the large number of male and female broodstock used for mass spawning breeding scheme in this species.

Tassanakajon *et al.* (1997) examined genetic diversity in 3 geographic samples of *P. monodon* (Trat and Angsila located in the Gulf of Thailand and Satun-Trang, located in the west of peninsular Thailand) using RAPD analysis of 5 primers and found that genetic distance within samples was 0.032-0.070. Conversely, higher diversity (0.171-0.199) was observed within each *S. scyllata*, *S. oceanuca* and *S. tranquebarica* (Klinbunga *et al.*, 2000).

Phylogenetic analysis of Thai abalone was carried out using a neighbor-joining approach. A neighbor-joining tree of three abalone indicated clear genetic separation between different species. All branches within a species revealed mononphyletic status in Thai abalone when analyzed with primers OPB11 and UBC101. Phylogeography was obvious in *H. ovina* (the Andaman and Gulf of Thailand samples) and partially observed *H. asinina* (Talibong and the Gulf samples) but not in *H. varia*.

The phylogenetic status of abalone in Thai waters was similar with that of cupped oysters in Thailand analyzed by 5 RAPD primers (OPA09, OPB01, OPB08, UBC210 and UBC220) where phylogeography was found in the white scar oyster (*C. belcheri*) and the black scar oyster (*C. iredalei*) but not in other species (*Saccostrea cucullata*, *S. forskali* and *Striostrea mytiloides*). However, clear differentiation of each species was found phylogenetically from RAPD analysis of mud crabs (*S. serrata*, *S. oceanica* and *S. tranquebarica*) in eastern Thailand. An intraspecific neighbor-joining tree of *H. asinina* based on 5 RAPD primers also revealed differentiation of this species.

The topology of a neighbor-joining tree between geographically different samples of three abalones in Thailand also indicated that *H. asinina* and *H. ovina* are more closely related one another than *H. varia*. This was contradictory to results from karyotyping of these abalone (Jarayabhand *et al.*, 1998) where *H. asinina* and *H. varia* were regarded as sister species. The parallel studies of this thesis based on PCR-RFLP of 16S and 18S rDNA and sequencing analysis of 16S rDNA using the same sample set (Pripue, 2001) confirms interspecific genetic relationships of Thai abalone determined by RAPD analysis.

Basically, three species of Thai abalone can be distinguished easily using external characteristics at the adult stage but taxonomic difficulties is found at the larval stages where they cannot be differentiated from each other (and possibly from other mollusc larvae). Therefore, identification of species-specific markers of abalone in Thailand is necessary for such application.

Using RAPD analysis, six *H. asinina*-specific RAPD markers were found from this study. In addition, RAPD markers specifically found in *H. asinina* originating from Talibong and the Philippines were also observed. Nevertheless, species-and/or population-specific markers were not found in *H. ovina* and *H.varia*. The species-specific markers found in this study may be used for quality control of the commercially traded seed. An inability to identify species-specific markers in those two species should be related to a limited number of RAPD primers used for genetic characterization of *H. ovina* and *H. varia*. Alternatively, the nature of genetic diversity in each abalone may have reflected the possibility to identify specific markers within species. In oysters, low genetic diversity (percentage of polymorphic bands and genetic distances) were found in three commercially cultured oysters; *C. belcheri*, *C. iredalei* and *S. cucullata* but extremely high diversity was observed in *S. forskali*, *S. mytiloides* and other morphologically unclear

oysters. Several species-specific RAPD markers were determined in the first group of oysters but not in the other group even though PCR-RFLP of three genes (16S, 18S and COI-COII) was additionally used. Comparing with oysters, *H. asinina* showed the pattern and the level of genetic diversity as similar as three commercially cultured oysters whereas *H. ovina* and *H. varia* exhibited similar genetic diversity and patterns as the second group of oysters. Specific markers could easily be identified from the former but not from the latter.

Nonetheless, RAPD markers may provided false negative results due to susceptibility of this technique on various components of PCR and quality of DNA examined. Therefore, these markers should be cloned and sequenced. Species-specific PCR can then be developed using newly designed forward and reverse primers. This Sequence Characterized Amplification Regions (SCAR) markers would eliminated problems arisen from inconsistency of RAPD markers.

More recently, molecular taxonomic key based on RAPD markers was constructed in three commercially important oysters; *C. belcheri*, *C. iredalei* and *S. cuculalata* (Klinbunga *et al.*, 2001). Three specie-specific SCAR markers were further developed by cloning and sequencing of *C. belcheri*-specific RAPD markers. A primer set was designed from clones representing each markers. Specificity and sensitivity tests indicated their species-specific nature that can detect DNA of the target species at approximately 30 pg.

RAPD-PCR has been successfully used to examine genetic diversity and population differentiation in several economically important marine species in Thailand including *P. monodon* (Tassanakajon *et al.*, 1997 and 1998), mud crabs (Klinbunga *et al.*, 2000) and oysters (Klinbunga *et al.*, 2001) and to develop SCAR markers in *C. belcheri*

(Klinbunga *et al.*, 2000). In the thesis, it has been used to identify species-specific markers in *H. asinina* and phylogeography of abalone in Thailand. Population differentiation of *H. asinina* and *H. varia* in Thailand suggests the existence of different stocks within a particular species which should be treated as separate management units. In the conservation point of view, transferring of different stocks to other habitat should be limited. Restocking stocking programs, if carried out, should be restricted to the local stock. For aquaculture application, heritability for economically important phenotypes (growth, mortality and disease-resistance) between different coastal regions of *H. asinina* (and possibly *H. ovina* if can be cultured) should be carried out. Consistent quality control for seed production and canned products of *H. asinina* can be performed after SCAR markers are further developed from RAPD markers found in this study.