CHAPTER V

DISCUSSION

Domestication and subsequently selective breeding programs (SBPs) are long-term processes (artificial and natural selection) used to improve commercially important trait in selected populations. Basically, short generation time and high fecundity of abalone suggested that genetic improvement of *H. asinina* is promising and can be done in a reasonable period of time.

To carry out effective breeding programs in *H. asinina*, high genetic diversity stocks should be established. Integrated knowledge on population genetics for estimation of genetic variation levels, molecular genetics for the identifying genetic markers at different levels and quantitative genetics for selection scheme and estimation of heritability for economically important phenotypes are required to improve the efficiency of genetic improvement in this species

Five microsatellite loci were used to investigate genetic diversity of *H. asinina* in hatchery and wild (Talibong Island) stocks. The average number of allele per locus of the hatchery samples in this study was 8.00 ± 3.67 for G8 and 8.40 ± 3.36 for B samples which were slightly lower than 12.00 ± 2.00, 8.33 ± 0.58 and 8.67 ± 7.23 in wild *H. asinina* from Cambodia, Samet Island and Trang analyzed at 3 microsatellite loci (Tang et al., 2005). The observed heterozygosity of the hatchery samples (0.69 ± 0.308 and 0.56 ± 0.241) was comparable with that of the wild stocks (0.62 ± 0.16, 0.78 ± 0.13 and 0.58 ± 0.28) even though the sample size of the hatchery abalone was larger than the wild stock previously reported by Tang et al. (2005). Accordingly, only slightly loss of genetic diversity was found in the subsequent generation of the hatchery samples.

Reduction in allelic diversity (i.e., number of alleles per locus) relative to wild populations seems to be a characteristic of hatchery organisms. The slightly reduction in genetic variation in these hatchery samples may be caused by suboptimal of parent abalone contributing in the previous generation increasing the effect of genetic drift, as has previously been reported for other hatchery-reared abalone (Li et al., 2004).

Apart from founder effects, artificial and natural selection in the culture environment might have changed the overall allelic composition of the farmed strain relative to wild population. Allelic frequency in wild populations is not always reflected in hatchery populations. The change in allele frequencies could be caused by non-random mating in the production of a new generation.

The hatchery samples (G8 and B) in this study have been propagated for eight generations. Relatively high levels of genetic diversity of these hatchery samples may result from the control breeding plans and crosses using the mass spawn approach. It is showing that the breeding plan carried out at present does not cause a significant effect with the thrashing of allele in the propagated abalone stock. Apparently, Sichang Marine Science Research and Training Station (SMaRT), Chulalongkorn University has maintained 8 stocks of *H. asinine* and all of them are currently used for production. As a result, genetic diversity of all culture stocks of *H. asinina* should be further studied. The genetic-based breeding plans of *H. asinina* can then be carried out more effectively.

Microsatellite DNA markers were used to investigate levels of genetic diversity within cultured populations of *Haliotis midae* and *Haliotis rubra* in South Africa and Australia, respectively. The cultured populations examined were F1 progeny of wild caught broodstock. The culture populations show a decline of the number of alleles per locus (35–62% allele loss) when compared to wild stocks in the area of respective broodstock collection. There was, however, no associated loss of heterozygosity. Changes in the frequency of alleles between farmed and wild samples were observed in both species. The estimated effective population size of *H. midae* broodstock was between 75.3 (SD ± 57.6) and 43.5 (± 29.8) for a west coast farm and between 18.5 (± 8.4) and 16.8 (± 8.0) for an east coast farm. The observed loss of alleles in both farm samples was significantly greater than that expected due to genetic drift based on such effective population size estimates. The effective population size of a farm sample of *H. rubra* was estimated at between 27.2 (± 3.8) and 22.4 (± 4.7). The observed loss of alleles in this instance was not significantly greater than expected due to genetic drift (Evans et al., 2004).

Likewise, genetic diversity in three hatchery strains and two wild stocks of Pacific abalone *Haliotis discus hannai* was examined using six microsatellite markers. High polymorphism at the microsatellite loci was found within both hatchery and wild abalone populations. The hatchery strains showed less genetic variation as revealed in lower number of alleles and lower expected heterozygosity, indicating that bottleneck effects occurred when each strain was founded (Li et al., 2004).

Hara and Sekino (2006) studied genetic variation of *H. discus hannai* within hatchery stocks were assayed with nine microsatelltie markers. The mean number of alleles per locus, observed and expected heterozygosity per locus were 4.5 - 8.3 alleles, 0.509 - 0.663 and 0.497 - 0.648, respectively. Results indicated comparable diversity levels *H. discus hannai* of *H. asinina* in this study.

In addition, loss of genetic diversity was also observed in the F2 of the Phillipine *H. asinina*. This hatchery sample was initially established from mass spawning of approximately 200 founders. The average number of alleles of this hatchery sample analyzed by *CUHas2*, *CUHas3* and *CUHas8* was only 3.67 ± 1.53 but the observed heterozygosity was relatively high (0.67 ± 0.58) (Tang et al., 2004)

Interestingly, non-overlapping alleles were observed at *CUHas8*: that is, the Talibong sample possessed smaller alleles (144 - 159 bp) than those detected in the hatchery sample (159 - 220 bp). Similar results on non-overlapping allele distribution at this locus were previously reported in abalone from the Talibong (Trang) and Samet samples (Tang et al., 2005). This suggested that *CUHas8* has potential to indicate the coastal origins of *H. asinina* founders of the hatchery samples.

The hatchery stocks at SMaRT were initially established from samples covering both coastal sides of Thai waters (Talibong and Samet Island). Therefore, genotyping of all maintained stocks should be carried out and wild specimens originating from Samet Island should be included for clearly illustrating that the present hatchery samples are majorly contributed by founders from Samet Island.

Recently, AFLP-derived markers indicating geographic origins of *H. asinina* in Thai waters were successfully developed. Three composite SSCP haplotypes (BBB, BAB and ABB) were found in 90, 5 and 5% of *H. asinina* from Talibong Island

(west, N = 20) whereas non-overlapping genotypes (AAA, DAA and CAA) were observed in that from Cambodia (60, 30 and 10%, N = 20). The hatchery-propagated *H. asinina*, initially established from mass spawning of founders from different geographic locations, only exhibited the east coast genotypes (AAA and CAA, 55/80) and its variants (AAC, 14/80). Like data from microsatellites, AFLP-derived markers implied that *H. asinina* from the east coast should be more adaptable to the cultivation than that from the west coast of Thailand (Prarichat Praipue, unpublished data).

In this study, genetic diversity of the Talibong sample was also evaluated but the sample size was limited. Considering the number of alleles per locus, a lower level of that genetic diversity index was observed in 4 microsatellite loci (*CUHas2, CUHas3, CUHas8* and *Haμ13*). To eliminate effects from the use of different sample sizes, the discrimination capacity was introduced and clearly indicated that the Talibong sample (DC = 0.349 ± 0.201) displayed a greater genetic diversity level than the hatchery samples. (DC = 0.175 ± 0.096 for G8 and 0.216 ± 0.133 for B).

Dong et al., (2005) carried out parentage assignment in the Chinese shrimp (*Fenneropenaeus chinensis*) using five microsatellites loci and 90.7% of the progeny were able to assign to one parental pair in a 30 factorial cross. Data simulation indicated that power of discrimination and power of exclusion indices were greater when one sire of parents is known. Although those parameters in this study were greater than their data, candidate parent genotypes were not known causing a significant reduction of the accuracy to carry out individuality and parentage analysis of *H. asinina*.

Significant differences between observed and expected heterozygosity resulting in of Hardy-Weinberg expectation were observed at *CUHas2 CUHas3* and *Haμ2J* ($P < 0.05$). As in this study, 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* (Tang et al., 2004 and 2005).

In artificial propagation programs, for instance breeding of *H. asinina*, individuality and family groups of propagated progeny reared in the large communal tank need to be determined and microsatellites are ideal for such purposes. Nevertheless, there are also limitations to their use that have recently come to light from studies of many different taxa of marine and terrestrial species. One frequently reported problem is the occurrence of null (non-amplifying) alleles usually caused by point mutations in the primer annealing sites (Callen et al., 1993). Null alleles at microsatellite loci have been found in many taxa (Callen et al., 1993 Ardren et al., 1999) and are common in abalone (Li et al., 2003). If null alleles are present, then a heterozygote bearing the null allele will be mistyped as a homozygote. This scoring error can cause a false observed heterozygote deficiency in the population and will result in the erroneous elimination of putative parents or errors in the degree of relatedness between individuals in kinship analysis (Ardren et al., 1999). Therefore, the performance of microsatellite markers should be evaluated before they are routinely employed and markers that do not exhibit Mendelian inheritance should be excluded.

It has been accepted that *H. asinina* has the fastest natural growth rate among abalone and reaches sexual maturity within one year. Accordingly, it is a suitable abalone species for selective breeding for commercially important traits such as rapid growth. The heritability for growth-related traits at 12 months of age by creating a single cohort of 84 families in a full-factorial mating design consisting of 14 sires and 6 dams was recently examined. Using an animal model, heritability estimates were 0.48 ± 0.15 for shell length, 0.38 ± 0.13 for shell width and 0.36 ± 0.13 for weight. Genetic correlations were >0.98 between shell parameters and weight, indicating that breeding for weight gains could be successfully achieved by selecting for shell length (Lucas et al., 2006).

Founder effects and genetic drift decrease the ancestral genetic variability of small populations, variability which is supposed to be essential for the initiation of domestication. An increase in inbreeding giving rise to inbreeding depression may lead to poorer performance and even to population extinction (Bierne et al., 2000).

In this study, a total number of 20 contributed individuals (10 males and 10 females) were inferred from the progeny genotypes. This data should be taken with cautions as the lack of parentage genotypes. Therefore, microsatellite patterns of potential sires and/or dams should be examined for more accurate assignment of parents.

Only N_e = 20 resulting in the inbreeding coefficient of 2.5% per generation were elucidated in the present study. Theoretically, N_e > 50 (with the inbreeding coefficient of 1.0% per generation) is required to maintain the long term genetic variability of the hatchery stocks (Gall, 1987; Allendorf and Ryman, 1987). Nevertheless, an occurrence of greater inbreeding coefficient than the recommended level per generation may not always resulting in reducing of performance for economically important traits unless inbreeding depression (e.g. increasing frequencies of deleterious alleles) is happened.

Results from this study suggested that N_e of the present stock should be increased in the subsequent generations. This can be carried out by increasing broodstock numbers and by undertaking several spawning between equal numbers of males and females and then combining the embryos to produce a 'mass' generation (Appleyard and Ward, 2006).

Jerry et al. (2006) determined the relative performance of 22 families of *P. japonicus* reared in commercial production ponds. Six thousands of postlarvae from each of 22 families, whose maternal parents had been genotyped at 8 microsatellite loci, were stocked into each of four 1 ha ponds. A total of 6000 individuals were harvested after 6 months and randomly weighed from each pond. Mean wet weight of the shrimp from one pond was significantly lower than that of the other three ponds demonstrating a possible pond effect on growth rate. The representation of families in the top 10% of each pond's weight distribution was then determined by randomly genotyping up to 300 individuals from this upper weight class. Parentage analyses based on individual genotypic data demonstrated that some families were over-represented in the top 10% in all ponds, while others were underrepresented due to slower growth rates.

The lack of parental genotypes of *H. asinina* limited the ability to effectively track the family genotypes of abalone in this study. Therefore, detection of families contributing the upper weight class using large sample sizes with replication was not possible. Neither a genetic linkage map nor the basic information about genes controlling growth was available in *H. asinina*. Accordingly, direct (and random) association between genotype and the body weight of the hatchery group B sample was examined.

The hatchery sample group B was divided into three subgroups (BL, BM and BS) according to the body weight. Genetic heterogeneity in allele distribution frequencies between pairs of subgroups were found between BL-BM at 3 microsatellites loci, between BL-BS at 3 microsatellites loci and between BM-BS at 2 microsatellites loci ($P < 0.05$). Genetic heterogeneity for all loci was significantly different for all pairwise comparisons ($P < 0.01$).

Association between genotypes and the body weight was initially tested at each locus using regression analysis. Only the locus *Haμ13* was significantly correlated between genotypes and the body weight ($P < 0.05$). Other loci did not show association between those parameters ($P < 0.05$). Notably, results from this study is only preliminary and the only possible way that genotypes of *Haμ13* significantly associated with the body weight of *H. asinina* is that that locus is located in vicinity with the QTL for growth in this species.

Mean bodyweight of abalone possessing homo- and heterozygotic status of the 128 allele was lower ($3.477 \pm 0.735 - 7.430 \pm 0.099$) than the average weight of the B sample (7.60 ± 2.758, $N = 280$). The representation of those genotypes in the slow growth *H. asinina* implied that some genotypes may predominate in lower or upper weight class.

Significant differences between the body weight of the B sample having different genotypes (e.g. between homozygotes and heterozygotes carrying the 124 alleles and those carrying the 128 allele) were found ($P < 0.05$). Therefore, breeding of founders carrying 124 allele (s) instead of 128 allele (s) should be considered for the subsequent generation of this hatchery stock. In *H. asinina*, females grow faster than males for approximately 25%. However, sexes of examined specimens were not

determined as a result the biased effects on different numbers of females and males in each genotype may influence the analysis.

In conclusions, levels of genetic diversity in the present generation and the number of contributed founders in the previous generation of the examined hatchery sample provided useful information to elevate management efficiency of breeding program of *H. asinina*. More appropriate breeding plans should be implemented to reduce the inbreeding coefficient of the hatchery stock. As a result, genetic diversity of the established stocks should be maximized and regularly monitored by highly polymorphic microsatellites. The possible correlation between microsatellite genotypes and a phenotype (i.e. the body weight) was only preliminary results and require further study but demonstrating the possibility to apply for selective breeding programs of *H. asinina*.