CHAPTER II

LITERATURE REVIEW

2.1 Taxonomy of *Haliotis asinina*

H. asinina is a univalve mollusk (Figure 2.1). The taxonomic definition of *H. asinina* is as followed (Hahn, 1989)

Phylum Mollusca

Class Gastropoda

Subclass Prosobranchia

Order Archeogastropoda

Suborder Zygobranchia

Family Haliotidae

Genus *Haliotis*

Species *asinina*

Scientific name: *Haliotis asinina* (Linnaeus, 1758)

Common name: Ass's Ear Shell (Eng.), Mimigai (Jap.)

2.2 Morphology and anatomy

- Shell

The shell is on the top and covers most of the abalone. The outside of its shell is greenish brown and rougher than the insides part which is smooth and pearly. It generally has an oval shape with the long anterior-posterior axis. The shell is in a spiral form like a common snail but quite flatter, about a fifth as high as it is long. Moreover, there is a row of holes found just in from the left-hand side of the shell, extending anteriorly to just over the head. The abalone shell grows as a spiral by the addition of new material on the anterior. The anterior holes are the biggest and those

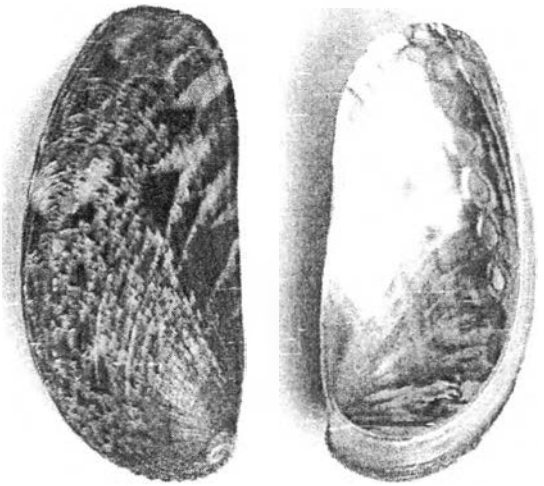

Figure 2.1 Tropical abalone *Haliotis asinina*.

(http://www.dkimages.com/discover/previews/860/20007256.JPG)

toward the back are usually blocked. The holes assist with respiration, removal wastes and release of gametes.

- Foot

The foot of an abalone is a large muscle used for crawling onto the seabed in typical snail-like manner. The foot is not suited to crawling over on clinging onto sand because in sandy places, the abalone can be easily turned over and makes easy food for predators. As a consequence, abalones are generally found only in areas of hard rock or coral. The part of the foot that is not completely covered by the shell looks similar to a pair of lips and is often referred to as such. Around the outside of the foot, extending from the upper part of the lips, is a series of tentacles, which presumably detect predators and food by touch and taste.

- Head

The head is located in front of the foot, which is typically snail-like with tentacles similar to those on the lip. The head tentacles are larger and similar to the eye stalks of land snails. The mouth is at the base of the head underneath the lips. It is a circular orifice in the middle of a circular piece of flesh called the oral disc. In the

mouth is a tongue-like organ covered with teeth called the radula, which is used to rasp food.

- Gills

The gill chamber is next to the mouth and under the respiratory pores. The sea water is drawn into the anterior of the mantle cavity and passed over the gills. Oxygen is taken up and waste gases are given off. The used water is passed out through the holes of the shell.

- Gut

Gut is hidden above the foot. The shell muscle, or stalk of the foot, extends up and attached to the middle of the shell. Gut coils around the space between the stalk of the foot and the rim of the shell.

- Reproductive glands

The reproductive glands or gonads envelop the tubes of the gut. They form a large cone-shaped appendage between the shell as the holes and the foot. Gonad is located on the same side of the shell and extends up into coiled apex of the shell. The gonad is green in ripe females and cream colored in ripe male. Usually, immature sex glands of either sex are gray. In mature, the gonad is clearly defined and swollen and is termed fat.

- Circulatory system

Abalone does have a heart on its left side and blood flows through the arteries, sinuses and veins, assisted by the surrounding tissues and muscles. Abalones blood contains a copper-based respiratory pigment, haemocyanin, which is blue when highly oxygenated but colorless when no oxygen is present. The abalone`s heart pumps oxygenated blood from gills into the foot along two central vessels which branch into smaller tubes. From the small tubes, blood and oxygen infiltrate into tissues. The blood then drains into another system of small tubes and moves back to a larger central cavity in the foot which carries it to gills to be oxygenated again.

Since abalone has no obvious brain structure, it is considered to be a primitive animal.

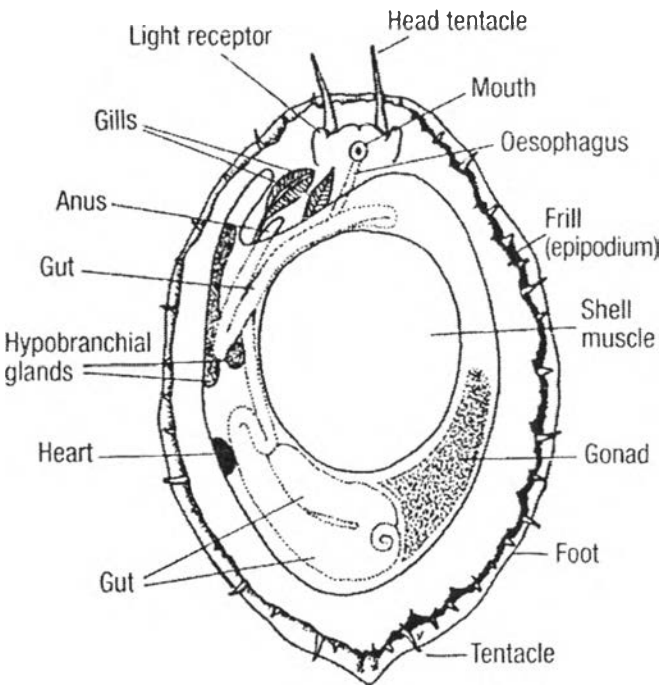

Figure 2.2 Ventral view anatomy of abalone

(http://www.diseasewatch.com/ documents/CD/index/images/ab-anatomy-1.jpg)

2.3 Life cycle

Abalones have separate sexes. Abalone eggs are externally fertilized. To reproduce, they broadcast sperm and eggs into the sea, relying on high gamete densities for successful fertilization, a reproductive strategy requiring densely aggregated adults for success.

When gametes are fused, the fertilized egg divides repeatedly and forms a larva. At the beginning abalone larvae are tiny and have no shell. The larvae go through a series of changes in body from. Initially, an upward swimming (trochophore) larvae are produced, probably as an adaptation to avoid predation by

benthic filter feeders. The larvae are free-swimming for only a few days.The larvae go through a series of changes in the body form to veliger stages (Figure 2.3). After about one week, the larvae sink to bottom where they cling to rocks and crevice with their single powerful foot.

Juvenile abalones feed on rock-encrusting coralline algae and on diatom and bacterial films. This process is called settlement and the developmental stage of abalone is termed spat. The abalone's body transforms into a miniature copy of adults. The minimum duration of larval stages is about 4 days but larval life duration would be expected to be much longer for most exploited abalone inhabiting cooler temperature waters.

Adult abalones feed primarily on loose pieces of marine algae drifting with the surge or current. After the gonads start to develop and the abalones become sexually mature and the cycle repeats itself. Almost abalones become mature between the first and third year. The life span of abalone is longer than a decade.

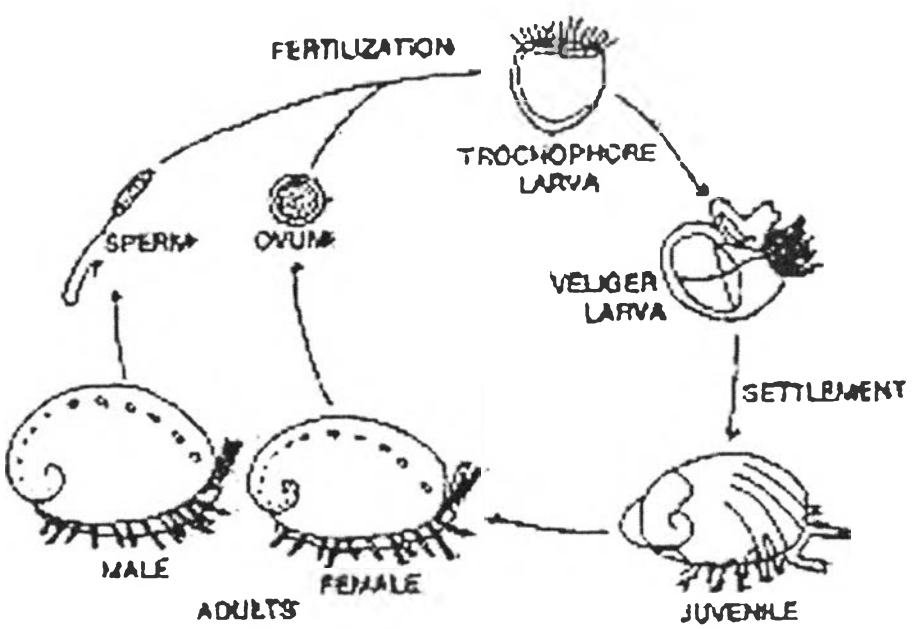

Figure 2.3 Life cycle of abalone (http://www.fishtech.com/facts.html)

2.4 Habitat and distributions

Abalones occur in all of the major oceans of the world but they are more abundantly distributed in the temperate zones. Generally, abalones prefer shallow, turbulent waters with high levels of dissolved oxygen and hard surfaces for settlement.

Primarily, abalone interacts with their immediate habitat with the foot. The abalone uses its foot to crawl from place to place in a typical snail-like manner. The foot of an abalone is not suited to crawling over or clinging onto sand. In sandy places, the abalone can be easily turned over and make easy food for predators. As a consequence, abalone is generally found only in areas of hard rock or coral. Abalones avoid the light so that in daylight, they are usually found hiding in crevices on rocky reefs and under rocky overhangs. The nature of their bodies constrains them to live in certain habitats and adopt appropriate modes of behavior in those habitats.

Geiger (2000) reported that *H. asinina* and *H. ovina* were found in more than 100 localities whereas *H. varia* Linnaeus, 1758 had the highest occurrence, 317 data points (Figure 1.2). Distribution of *H. asinina* has been reported in South East Asia, Japan and Australia but *H. ovina* and *H. varia* are wider distributed. Their geographic distribution includes the Indian Ocean and the East Coast of Africa.

In Thailand, *H. ovina* had been reported to be more common than *H. asinina* along the upper eastern Gulf of Thailand (Kakhai and Petjamrat, 1992), even though Sungthong et al. (1991) found that *H. asinina* is more common at Samet Island (Rayong , eastern Thailand . Only small numbers of *H. ovina* have been found along the lower eastern coast. There has been no report on the finding of *H. varia* in the Gulf of Thailand but a few literatures reported their availability in Peninsular Malaysia (Geiger, 1999 and 2000). Along the Andaman coastline, the relative abundance of each species was 81%, 17.3% and 1.7% for *H. varia*, *H. ovina* and for *H. asinina*, respectively (Jarayabhand and Paphavasit, 1996).

2.5 Molecular genetic techniques used in the thesis

2.5.1 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a technique widely used in molecular biology introduced by Mullis et al. (1987). With PCR, it is possible to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating millions or more copies of the DNA piece. PCR can be performed without restrictions on the form of DNA, and it can be extensively modified to perform a wide array of genetic manipulations.

PCR employs a heat-stable DNA polymerase such as *Taq* DNA polymerase. This DNA polymerase enzymatically assembles a new DNA strand from DNA building blocks, the nucleotides, using single-stranded DNA as template and DNA oligonucleotides (primers) required for initiation of DNA synthesis. PCR methods use thermal cycling (repeated heating and cooling) of the reaction to a defined series of temperature steps. These different temperature steps are necessary to bring about physical separation of the strands in a DNA double helix (DNA melting), and permit DNA synthesis by the DNA polymerase to selectively amplify the target DNA.

The power and selectivity of PCR are primarily due to selecting primers that are highly complementary to the DNA region targeted for amplification, and to the thermal cycling conditions used. PCR is used to amplify specific regions of a DNA strand (the DNA target). This can be a single gene, a part of a gene, or a non-coding sequence (Cheng *et al.*, 1994).

The PCR reaction requires several components and reagents. These components include DNA template that contains the DNA region (target) to be amplified, a pair of primers which are complementary to the DNA regions at the 5' and 3' ends of the DNA region, Deoxynucleoside triphosphates (dNTPs; dATP, dCTP, dGTP and dTTP) which is used by the DNA polymerases to synthesize a new DNA strand, buffer solution providing a suitable chemical environment for optimum activity and stability of the DNA polymerase and DNA polymerase such as *Taq polymerase* or another DNA polymerase (Sambrook and Russel, 2001).

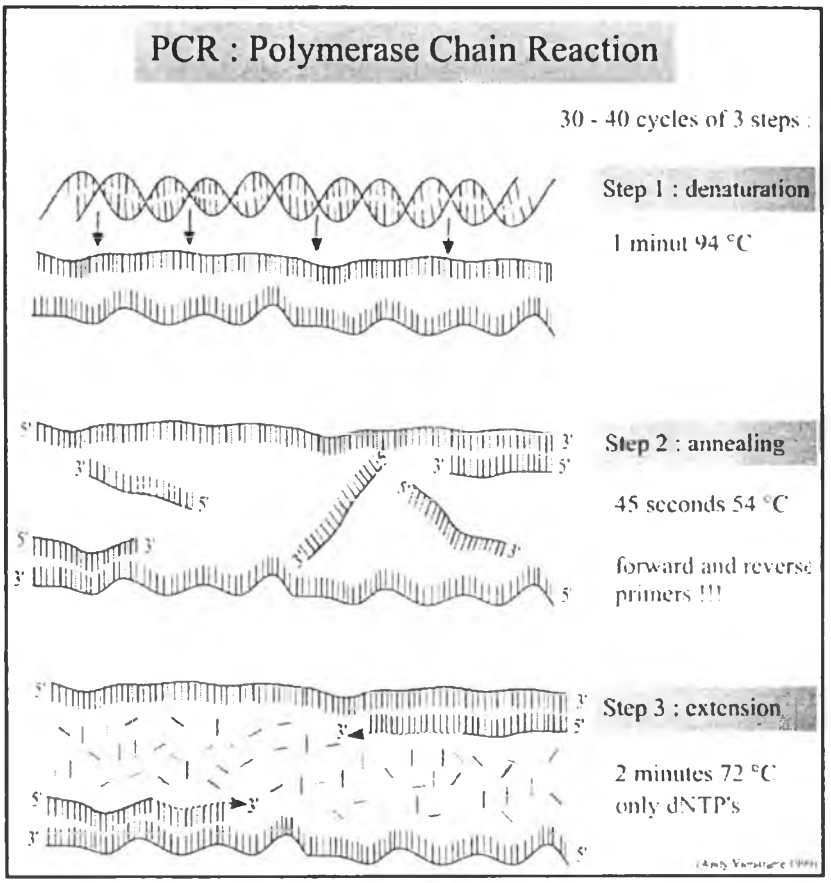

Figure 2.4 General illustration of PCR.
(http://users.ugent.be/~avierstr/principles/pcrsteps.gif)

The amplification reaction consists of three major steps; a denaturation step of double stranded DNA at high temperature, an annealing step to allow forming of hybrid primers-template at the optimal temperature, and an extension step of the annealed primers by the heat-stable DNA polymerase. The cycles are repeated for 30-40 times (Figure 2.4). The amplification product is usually determined by electrophoresis.

2.5.2 Microsatellites

Microsatellites, also called simple sequence repeats (SSR) or short tandem repeats (STR) DNA, are tandem arrays containing short DNA consisting of short repeats motifs of 1-6 nucleotides (e.g., ACA or GATA) which arrayed in tandem repeated manner for approximately 10-50 copies (Hearne et al., 1992).

Microsatellites are highly abundant and randomly dispersed in most eukaryote genomes. It was estimated that one microsatellite locus may be found every 10 kb in eukaryotic genomes. Microsatellites are embedded in untranslated genomic DNA sequences (Type II) or the coding region of genes (Type I) (Valdes et al., 1993). Due mainly to high mutation rate of the microsatellite loci (10^{-6} to 10^{-12} per generation), microsatellites are powerful for detection of genetic diversity of eukaryotes.

Each microsatellite locus is flanked by a unique sequence. As a result, locus-specific primers can be synthesized complementary to the flanking sequences and PCR is then used to amplify the tandem array of the microsatellite locus. The PCR-amplified products are size-fractionated on denaturing polyacrylamide gels. The abundance of di-nucleotide microsatellite in eukaryotic genomes make them, in general, easily isolated by standard cloning methodologies.

Type II microsatellite primers can be developed by cloning random segments of DNA from the focal species. Genomic DNA isolated from the target species is separately digested by usually tetranucleotide recognizing endonucleases (*Pal* I, *Rsa* I, *Sau* 3AI, etc.). The restricted DNA is size-fractionated by agarose gel electrophoresis. Restriction fragments of 300-800 bp are excised from the agarose gel, purified and cloned into an appropriate plasmid vector such as pUC18. After transformation and selection, the constructed library is then screened using radiolabeled (or fluorescently labeled) oligonucleotide corresponding to common microsatellite repeats such as $(GT)_n$ or $(CA)_n$. Double stranded DNA from positively hybridized colonies is sequenced in both direction. Once the sequence of a microsatellite locus is known, locus-specific primers are then designed from non-repetitive flanking region. PCR of such a locus can then carry out (Queller et al., 1993).

For species that a large number of EST is available, type I microsatellite primers can be designed from cDNA containing simple sequence repeats. The use of type I microsatellites for genetic diversity studies have been reported in seveal species as the data on EST sequences of various species have accumulated rapidly. However, the disadvantage on the use of type I microsatellites for population genetic studies is that they may be selective non-neutrally. Therefore, type I microsatellites should be carefully tested before applied for that apllication.

The polymorphism of microsatellites varies greatly among loci but is usually higher than that of allozymes and mtDNA (Wright and Bentzen, 1994). The range of polymorphism allows for the versatility necessary to select suitable loci for a particular application. For example, highly polymorphic loci exhibiting large numbers of alleles are ideal for gene mapping and pedigree analysis, whereas less polymorphic loci can be used to analyze population differentiation (O'Reilly and Wright, 1995).

When multiple loci of highly polymorphic microsatellites are used for genotyping, the information enabling the distinction between specific individuals can be obtained (Wright and Bentzen, 1995). Moreover, co-dominant Mendelian inheritance makes microsatellites more informative in pedigree studies and suitable for large breeding programs than other molecular markers that segregate dominantly.

2.6 Applications of microsatellites

2.6.1 Applications of microsatellites for genetics and fisheries management

Beheregaray et al. (2004) isolated and characterized microsatellites from the pencilfish (*Nannostomus unifasciatus*). Nine loci were successfully amplified and screened for variation in the fish sample ($N = 30$) collected from Rios Tea, Cuiuni and Igarapé Arixaná (middle Rio Negro, Brazil). The number of alleles per locus and expected heterozygosity ranged from 4 to 19 and 0.39 to 0.86. Most loci were in Hardy–Weinberg equilibrium in all populations. However, Nu11 and Nu44 exhibited homozygote excesses possibly related to null alleles.

Donald and Spencer (2006) characterized microsatellites in the New Zealand mudflat topshell (*Diloma subrostrata*) and polymorphic at 5 loci (*DisuA3, DisuA8, DisuB12, DisuD4* and *DisuA5*) was preliminary examined. The number of alleles per locus ranged from 2 to 23, observed and expected levels of heterozygosity were 0.47-0.88 and 0.37-0.95, respectively. The observed and expected heterozygosities did not deviate from Hardy–Weinberg equilibrium ($P<0.05$). These markers are currently being used to investigate genetic population structure of *D. subrostrata* in New Zealand.

Pan et al. (2004) isolated 23 polymorphic microsatellites in the giant tiger shrimp, *Penaeus monodon* and examined genetic diversity of shrimp from three disparate localities in Taiwanese waters (N = 30). The number of alleles per locus ranged from 15 to 31. The observed and expected heterozygosities ranged from 0.4 to 0.933 and from 0.789 to 0.972, respectively. Genotypes of all loci deviated from Hardy–Weinberg expectation ($P < 0.05$).

Schrey and Heist (2003) examined population genetic structure of the shortfin mako shark (*Isurus oxyrinchus*) collected from the North Atlantic, South Atlantic, North Pacific, South Pacific, and South Africa (N = 432) using 4 microsatellite loci (*Iox-01, Iox-10, Iox-12* and *Iox-30*). The number of alleles per locus ranged from 14 to 46 and observed heterozygosities were 0.77 to 0.91. The estimates of F_{ST} were low and not statistically significant. The multilocus F_{ST} was 0.0014 ($P = 0.1292$) and individual locus F_{ST} results ranged from –0.002 to 0.0029 with no estimate being statistically significant (Iox-10, $P < 0.0001$). Results revealed the lack of intraspecific population differentiation in *I. oxyrinchus*.

Enriquez et al. (1999) compared the genetic diversity of a hatchery-reared stock of red sea bream (*Pagrus major*) (N = 200) used for stock enhancement with that of their broodstock (N = 248) using 4 microsatellite DNA markers (*Pma 1, Pma 3, Pma 4* and *Pma 5*). Their pedigree was also traced. The effective number of contributing parents (N_e) and inbreeding coefficient were estimated. It was found that the genetic diversity of the hatchery-reared stock in terms of the observed heterozygosity (H_o = 0.856) was not significant different ($P > 0.05$) than that of the broodstock (H_o = 0.841). The number of alleles per locus of hatchery-reared stock and broodstock were ranged from 14 - 33 and 21 - 48, respectively. Significant differences ($P < 0.05$) were found in the number of alleles per locus and in the frequencies among some major alleles of the two stocks. The estimated N_e was 63.7 individuals resulting in the estimated inbreeding coefficient of less than 0.8%. The results did not evident a loss of genetic variation and severe inbreeding of the hatchery-reared stock.

2.6.2 Applications of microsatellite for domestication and breeding programs

Taris et al. (2005) carried out parentage assignment of larvae (N = 1315) or adults (133, females and 10 males) of the Pacific oyster (*Crassostrea gigas*) using 3 microsatellite loci (*CG49, CG108* and *L10*). The parentage assignment was successfully applied for 93% (1224 out of 1318) of the progeny in a factorial cross between 3 females and 10 males. The inability to genotype the remaining 7% was due to DNA degradation of larvae rather than assignment uncertainty.

Dong et al. (2006) assessed the feasibility of parentage determination in aquaculture populations for the Chinese shrimp (*Fenneropenaeus chinensis*) using 5 microsatellite markers (*RS0683. EN0033, RS062. RS0622* and *RS1101*). In total, 226 individuals were genotyped, including 215 offspring, 6 candidate mothers and 5 candidate fathers. Number of alleles detected from five microsatellite loci ranged from 10 to 16. The mean expected heterozygosity was 0.875. Hardy–Weinberg test results indicated three (*RS062, RS0622* and *RS1101*) out of five markers did not follow Hardy–Weinberg equilibrium. Out of 215 offspring, 90.7% was assigned to their parental pairs exclusively in mixed families groups. When marker data from 5 loci were combined, the assignment success of progeny to their true parental increased to 92.9% in parentage identification with known parental and familial information. Results indicated that the use of microsatellite markers represents a realistic and effective alternative to physical tagging in a selection program and it allows the identification of parental effects on offspring performances from early life stages.

Palti et al. (2006) evaluated genotype × feed interactions in a commercial strain of the rainbow trout (*Oncorhynchus mykiss*). Eighteen microsatellite DNA markers were used to determine the pedigree of the top 1% and bottom 1% of progeny in a large scale commercial growth trial of 24,000 rainbow trout from 20 full-sib families (20 dams×10 sires in a nested mating design).The progeny were pooled at eyed stage and divided into 2 groups. Half of the fish from each family was fed a standard fishmeal-based diet and the other half was fed a plant protein (gluten)-based diet to determine the relative family rankings in each diet. Large genetic variation for growth was identified (high, intermediate and low) for both diets and the sire effect was found to be highly significant ($P < 0.001$). A significant genotype × diet interaction was not observed in this study, which suggests that fish that grow faster

when fed fishmeal diets are likely to grow faster when fed the particular gluten-based feed, and therefore current commercial strains that exhibit superior growth should retain their improved performance if raised on gluten-based diets. Error rates for parentage determination with the 5, 6, 8, 10 and 18 most informative markers were 17.5%, 7.5%, 6.25%, 5.00% and 3.75%, respectively. Multiplexing microsatellite markers would further improve the efficiency of parentage assignment protocols in large-scale rainbow trout selection programs.

2.6.3 Genetic studies of abalone using microsatellites

An and Han (2006) developed 17 microsatellite markers in *H. discus hannai*. All loci were found to be polymorphic with an average of 13.1 alleles per locus (range 3 – 28). The mean observed and expected heterozygosities were 0.77 (range 0.17 – 1.00) and 0.79 (range 0.42 – 0.96), respectively. Six loci deviated significantly from Hardy–Weinberg equilibrium (*KHdh46, KHdh50, KHdh53, KHdh55, KHdh57* and *KHdh64*), and thus should be used with caution. The high polymorphic levels of these microsatellites suggest that they should provide useful markers for studies of trait mapping, kinship and population genetics.

Sekino and Hara (2001) used 5 microsatellite loci (*Hdd6C, Hdd108C, Hdd114B, Hdd115B* and *Hdd229*) to estimate genetic variability of the Pacific abalone *H. discus discus*. All five loci were successfully amplified with polymorphism. The genetic variability varied depending on the locus, the number of alleles ranged from 3 to 10, and the observed and expected heterozygosity ranged from 0.17 to 0.80, and 0.20 to 0.89, respectively. Two loci, *Hdd114B* and *Hdd229*, showed significant deviations of the observed genotype frequencies from Hardy–Weinberg's expectations ($P < 0.05$).

Miller et al. (2001) used 12 microsatellite markers in a population survey of abalone (*H. kamtschatkana*) from the coast of British Columbia. The survey included > 400 abalone collected from seven sites ranging from 20 to 90 kilometers apart. The number of alleles identified at each of the loci ranged from 20 to 63. Observed heterozygosities ranged from 0.44 to 0.93, Expected heterozygosities ranged from 0.68 to 0.96. Significant deviations from Hardy-Weinberg equilibrium were observed for six of the loci (*Hka*3, *Hka* 6, *Hka* 28, *Hka* 48, *Hka* 65 and *Hka* 85). The

polymorphism at microsatellite loci is useful in pedigree, QTL, management plan and population analyses.

Conod et al. (2002) examined genetic variation in five geographically isolated samples of the blacklip abalone, *H. rubra*, from south-eastern Australia by using RFLP analysis on the ND3/COIII region of mitochondrial DNA and five independent nuclear DNA microsatellite loci. They found that both techniques showed similar resolving power. They recommended that microsatellite DNA analysis is the preferred molecular technique for the fine scale investigation of blacklip abalone population structure because it makes possible the examination of numerous independent loci with potentially high levels of polymorphism. Both sample and locus specific homozygote excesses were recorded for the microsatellite loci. The most likely explanation for the locus specific deviations from Hardy-Weinberg expectations is the presence of null alleles.

Li et al. (2004) used 6 microsatellite markers (*Hdh1321, Hdd114B, Hdd108C, Hdh513, Hdh57* and *Hdh145*) to estimate the level of genetic diversity of *H. discus hannai* within three hatchery strains and two wild populations of Pacific abalone, and compared the degree of genetic differentiation between them. The 6 microsatellite loci were all highly polymorphic, while the degree of variability was different at each locus. *Hdh513* had the highest number of alleles (45) in the wild, Iwate Prefecture population (IKI), while the maximum number of alleles at *Hdh1321, Hdh57, Hdh145, Hdd114B*, and *Hdd108C* was 42, 11, 8, 29, and 6, respectively. The average number of alleles per locus in the hatchery and wild populations (5.4 and 22.4), the mean number of alleles per locus detected in the three hatchery strains ranged from 3.5 to 8.5, significantly lower than those in the two wild populations (21.8 and 23.0). The mean expected heterozygosity was 0.793 in both of the wild populations and ranged from 0.559 to 0.715 in the hatchery strains. Compared to the wild populations, expected heterozygosities were significantly reduced in the hatchery strains ($P < 0.05$). The results showing differentiation was found between the hatchery strains (F_{ST} range 0.243 – 0.427) and between the hatchery strains and wild populations (F_{ST} range 0.059 – 0.252) and no obvious difference was detected between the wild populations ($F_{ST} = 0.004$). The reduction in genetic variation in these hatchery strains

may be caused by a small number of parent abalone increasing the effect of genetic drift, as has previously been reported for other hatchery-reared broodstocks.

Li et al. (2003) studied the mode of inheritance of 7 microsatellite loci of *H. discus hannai* in 4 families with a reciprocal cross of 2 females x 2 males. All loci segregated codominantly, but only 3 loci (*Hdh1321, Hdh78,* and *Hdd108C*) conformed to Mendelian segregation and can be used for parental analysis and population genetic studies. When null alleles were considered, 2 loci (*Hdh1761* and *Hdh1457*) confirmed Mendelian expectations in all families, while the remaining 2 loci (*Hdd114B* and *Hdd229*) showed deviation from Mendelian segregation in at least one family even though null alleles were considered.

Selvamani et al. (2000) isolated and characterized 11 microsatellites in *H. asinina* and the genetic variability of these microsatellites was only examined in the Heron reef population of Australia (N = 21–41 per locus). The numbers of alleles per locus ranged from 2 to 25 alleles and the expected heterozygosity between 0.29 to 0.96. The level of allelic variability for all except two loci (Haμ13 and Haμ2K) was high. Two loci (*Haμ2J* and *Haμ2L*) significantly deviated from Hardy-Weinberg expectations owing to heterozygote deficiencies (P = 0.003 and P = $< 10^{-6}$, respectively).

Tang et al., (2004) developed polymorphic microsatellites to assess the genetic structure of *H. asinina* in Thailand. Microsatellites of *H. asinina* were isolated from 3 partial genomic libraries that were constructed using different approaches. Ten primer pairs (*CUHas1–CUHas10*) were analyzed to evaluate their polymorphic level. The numbers of alleles per locus, observed heterozygosity (H_o), and expected heterozygosity (H_e) ranged from 3 to 26 alleles, and 0.27 to 0.85 and 0.24 to 0.93, respectively. Three microsatellite loci (*CUHas2, CUHas3,* and *CUHas8*) were further used for examination of genetic diversity and differentiation of natural *H. asinina* in coastal waters of Thailand. Genetic variation in terms of the effective number of alleles (n_e), H_o, and H_e were higher in 2 samples from the Gulf of Thailand (n_e = 9.37, 7.66; H_o = 0.62, 0.78; and H_e = 0.87, 0.86) than those of a sample (n_e = 6.04; H_o = 0.58; and H_e = 0.62) from the Andaman Sea. Assessment of genetic heterogeneity, including allele frequency comparison and pairwise F_{ST} analysis, indicated

interpopulational differentiation, between natural *H. asinina* from the Gulf of Thailand and the Andaman Sea ($P < 0.0001$).

Tang et al., (2005) examined genetic heterogeneity of *H. asinina* of within the Gulf of Thailand and the Andaman Sea using microsatellite analysis. Three microsatellite loci (*CUHas1, CUHas4* and *CUHas5*) indicated relatively high genetic diversity in *H. asinina* (total number of alleles = 26, 5, 23, effective alleles per locus = 13.93, 2.47, 10.70 and observed heterozygosity = 0.84, 0.42 and 0.33, respectively). Significant population differentiation was also found between samples from different coastal regions ($P < 0.0001$). This further confirmed that the gene pool of natural *H. asinina* in coastal Thai waters can be genetically divided to 2 different populations; the Gulf of Thailand and the Andaman Sea.

Selvamani et al. (2001) used microsatellite DNA markers for parentage assignment of *H. asinina* larvae generated from 3 separate crosses by 2 highly polymorphic loci (*Haμ13* and *Haμ2K*) and 3 moderately polymorphic loci (*Haμ10, Haμ2J* and *Haμ3E*). The numbers of alleles per locus ranged from 12 to 25 and the expected heterozygosity was between 0.77 - 0.96. In all cases, the parents of an individual veliger could be determined from as few as 3 loci.

Lucas et al. (2006) estimated heritability for growth-related traits at 12 months of age of *H. asinina* by creating a single cohort of 84 families in a full-factorial mating design consisting of 14 sires and 6 dams. Of 500 progeny sampled, 89% were successfully assigned to their parents based on shared alleles at 5 polymorphic microsatellite loci (*Haμ2J, Haμ10, Haμ13, Haμ2K* and *Haμ3E*). Heritability estimates for shell length, shell width and weight were 0.48 ± 0.15, 0.38 ± 0.13 and 0.36 ± 0.13 respectively. 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. The present study suggests that microsatellite technology can facilitate the recovery of families each generation.