

Review

Aquaculture-oriented genetic researches in abalone: Current status and future perspective

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Basic genetic and cytogenetic information including polymorphic DNA markers, chromosomes and genome size was summarized to get insights into phylogenetic or systematic relationship among abalone species belonging to the genus *Haliotis*. Hybridization, triploidization and genetic mapping were also briefly reviewed as aquaculture-oriented genetic techniques to improve growth and other commercially important traits. Cryopreservation and other biotechnologies potentially applicable on genetic improvement were also briefly mentioned as supporting tools for efficient breeding of abalone strains.

Key words: Chromosome, DNA markers, genetic map, genome size, *Haliotis*, hybrid, polyploidy, selection

INTRODUCTION

Abalone species in the genus *Haliotis* with relatively large body size are distributed in the temperate zone including Japan, Australia, New Zealand, USA, Mexico and South Africa (Elliott, 2000; Hara, 2008a, b). In tropical and subtropical regions, small or 'cocktail size' *Haliotis* species are also popular (Jarayabhand and Paphavasit, 1996). Abalone species are commercially important because they have large edible part (adductor muscle or foot), that is, highly appreciated and reaching good values on the international market (Oakes and Ponte, 1996; Elliott, 2000). Thus, abalone species have been exploited by capture fisheries and natural stocks were overexploited. Such exploitation led to many conservation strategies including re-stocking, hatchery production and release of artificial seedlings. However, such a kind of semi-natural production is not considered so successful. Pacific abalone *Haliotis discus hannai* is an economically important species in Japan and stable propagation techniques have been established and stocking of artificial abalone seedlings has begun since 1970's (Hara, 2008a, b). Recently, more than 20 million seedlings are released every year so as to rehabilitate natural resources in the shallow sub tidal zone, but the annual production still remains approximately 2000 metric tones in Japan (Hara,

2008a, b). Survival and fecundity of released abalone are closely related to successful rehabilitation of wild resources by re-stocking. The above-mentioned example is also in agreement with FAO (2010) regarding the global fisheries production, suggesting that other sustainable production systems are necessary. On the other hand, there are many environmental and socio-economical issues that hinder the sustainable production of coastal wild stock.

Aquaculture of abalone is considered to have big potential as a sea-food farming industry. The slow growth in abalone species is considered the most serious problems (Elliott, 2000). Thus, growth is the most important aquaculture trait in the case of abalone farming, because faster growth to the marketable size reduces the production time and cost as the methods to improve growth and other commercially important traits of aquaculture abalone populations, hybridization, triploidization and genetic mapping have been practiced. Here, we briefly reviewed the current status and future perspective of abalone genetics with special references to aquaculture practices with basic biological information of *Haliotis* species.

BASIC GENETICS AND CYTOGENETICS

About 70 *Haliotis* species are distributed from the sub frigid zone to the tropical zone and about 20 to 30 species

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species have been utilized as fresh, frozen, and processed foods as well as materials for industrial arts (Elliott, 2000; Hara, 2008a, b). Taxonomy and systematics based on morphology are generally difficult and inconclusive in abalone species. Recent molecular genetic approach using hemocyanin sequence suggests the presence of genetically two distinct groups (Streit et al., 2006). The first group includes European *Haliotis tuberculata*, South African *Haliotis midae*, Indo-Pacific *Haliotis asinina*, Japanese *Haliotis diversicolor diversicolor*, and Taiwanese *Haliotis d. supertexta*. The second group includes East Pacific *Haliotis fulgens*, *Haliotis corrugate*, *Haliotis wallanlensis*, *Haliotis rufescens*, *Haliotis cracherodii*, Japanese *H. discus hannai*, and *Haliotis gigantea*. The other genetic studies indicate that the following two abalone species are possibly the same single one: Northwest Pacific *Haliotis madaka* and *H. d. hannai*, Indo-Pacific *H. diversicolor aquatilis* and *H. d. supertexta*, and Mediterranean *H. tuberculata* and *H. lamellose*, based on molecular data from the sperm lysin gene (Lee and Vacquiere, 1995), 18S rDNA (Naganuma et al., 1998), and 16S rDNA (An et al., 2005). These results roughly provide us with a phylogenetic inter-specific relationship among abalone species in the genus *Haliotis*.

Recent progress in molecular biology makes it possible to develop a large number of polymorphic DNA markers from nuclear genome of marine invertebrates. Microsatellite (MS) markers, as the most convenient co-dominant markers, were isolated and characterized by Sekino and Hara (2001), Sekino et al. (2005) and Li et al. (2002). Hara and Sekino (2005) genetically distinguished two resemble abalones, *H. d. discus* and *H. d. hannai* using MS markers. They also succeeded in showing clear inter-specific relationship among *H. gigantea*, *H. madaka*, *H. d. discus* and *H. d. hannai* by MS analyses (Sekino and Hara, 2007a). Luo et al. (2010) identified inter-specific hybrids between *H. d. hannai* and *H. gigantea* using amplified fragment-length polymorphism (AFLP) and MS. Hybrid identification by MS was also reported by Ibarra et al. (2005).

Cytogenetic results categorize abalone species into three groups based on chromosome numbers and karyotypes (Table 1). European species, *H. tuberculata* has $2n=28$, while Indo-Pacific (or Tropical and Sub-tropical) species, *H. varia*, *H. planate*, *H. diversicolor diversicolor*, *H. d. aquatilis*, *H. asinina* and *H. ovina*, show $2n=32$. In contrast, North East and North West Pacific species, *H. madaka*, *H. discus hannai*, *H. d. discus*, *H. gigantea*, *H. cracherodii*, *H. rufescens*, *H. fulgens*, and *H. corrugate* show $2n=36$. Very recently, it was reported that African Indo-Pacific species, *H. midae*, has 36 chromosomes (Van der Merwe and Roodt-Wilding, 2008; Franchini et al., 2010).

Using the difference in karyotype between the parental species, identification of hybrid abalone was also cytogenetically conducted (Amar-Basulto et al., 2011). At present, however, molecular cytogenetic studies including

chromosome banding and fluorescence *in situ* hybridization (FISH) have been poorly developed (Okumura et al., 1999; Gallardo-Escarate et al., 2005) and further detailed investigation is prerequisite to bridge near future linkage map and physical map.

There is a little knowledge about the genome size of abalone. C-value (genome size or cellular DNA content per haploid genome) was reported as approximately 2.14 pg in *H. corrugata*, 1.82 pg in *H. rufescens*, and 1.71 pg in *H. fulgens* (Gallardo-Escarate and Del Rio-Portilla, 2007), 1.43 pg in *H. midae* (Franchini et al., 2010), 1.84 pg in *H. d. hannai* and 1.45 pg in *H. d. aquatilis* (Adachi and Okumura, 2012).

HYBRIDIZATION

In hybrids, heterosis or hybrid vigor can be expected to improve traits such as growth. In Japan, artificial cross-breeding was performed in all the possible combination among *H. d. hannai*, *H. gigantea* and *H. madaka* (Ahmed et al., 2008). All hybridizing combinations gave viable progeny with true hybrid characteristics which were verified by allozyme genotypes and mtDNA restriction fragment length polymorphism (RFLPs); however lower fertilization rates than pure homospecific crosses were recorded. These hybrids exhibited normal or quasinormal gonad development similar to the parental species and they spawned fertile gametes. These hybrid abalones were reported to produce viable progeny by hybrid-hybrid F2 crosses and back crosses. In Mexican abalones, genetic certification was made on putative *H. fulgens* x *H. rufescens* hybrid abalone individuals by allozyme and microsatellite DNA analyses and then gonad development was examined in 'true' hybrid individuals (Ibarra et al., 2005). These hybrid males exhibited partial or total sterility because they showed development of spermatozoa and very few spermatozoa, but hybrid females showed vitellogenic or previtellogenic oocytes. These results suggest that fertile inter-specific hybrid abalone should arise from the cross-breeding between closely related species such as *H. discus discus* - *H. d. hannai* - *H. madaka* - *H. gigantea* combinations, and semi-fertile (male-sterile) hybrid abalones from *H. fulgens* - *H. rufescens* combination. However, reproductive capacity is not fully understood for hybrids between remotely related species due to the shortage of hybrid studies.

On the other hand, Koike et al. (1988) reported that growth rates of F1 hybrids (*H. madaka* female x *H. gigantea* male, *H. gigantea* female x *H. madaka* male, *H. gigantea* female x *H. discus* male, and *H. madaka* female x *H. discus* male) were better than their parental species in 6-month-old juvenile stages. They also reported that daily feeding rates and monthly growth rates were superior in the hybrid *H. madaka* female x *H. gigantea* male. The outperformance in growth was also reported in *H. gigantea* female x *H. discus discus* male hybrid group

Table 1. Diploid chromosome numbers and karyotypes reported in abalone species.

Distribution <i>Haliotis</i> Species	Chromosome number 2n	Karyotype						NOR or rDNA	Reference
		M	M/SM	SM	SM/ST	ST	T		
European									
<i>H. tuberculata</i>	28	16		12					Arai and Wilkins (1986)
	28a								Colombera and Tagliaferri (1983)
<i>H. lamellose</i>	28a								Colombera and Tagliaferri (1983)
Indo-Pacific									
<i>H. varia</i>	32	26		6					Nakamura (1985)
	32	18		12	2				Arai et al. (1988)
	32	16		16					Jarayabhand et al. (1998)
<i>H. planata</i>	32	18		12	2				Arai et al. (1988)
<i>H. diversicolor</i>	32	16		14		2			Arai et al. (1988)
<i>H. d. aquatilis</i>	32	16		10	4	2			Nakamura (1985)
<i>H. asinina</i>	32	20		12					Jarayabhand et al. (1998)
<i>H. ovina</i>	32	18		12			2		Jarayabhand et al. (1998)
<i>H. midae</i>	36								Van der Merwe and Roodt-Wilding (2008)
	36	6	1	9		2			Franchini et al. (2010)
Pacific Northwest									
<i>H. discus discus</i>	36	20		16					Arai et al. (1982)
	36	20		16					Miyaki et al. (1997)
<i>H. d. hannai</i>	36	20		16					Arai et al. (1982)
	36	22		14					Wang et al. (1988)
	36	22		14				*	Okumura et al. (1999)
<i>H. madaka</i>	36	20		16					Miyaki et al. (1999)
<i>H. gigantea</i>	36a								Nakamura (1986)
	36	20		16					Miyaki et al. (1997)
Pacific Northeast									
<i>H. cracherodii</i>	36	16		16		4			Minkler (1977)
	36	16		16		4			Gallardo-Escarate and Del Rio-Portilla (2007)
<i>H. fulgens</i>	36	20	6	8	2				Hernandez-Ibarra et al. (2004)
	36	16		16		4		**	Gallardo-Escarate et al. (2005)
<i>H. corrugata</i>	36	20		14		2		***	Gallardo-Escarate et al. (2005)
	36	20		14		2			Gallardo-Escarate and Del Rio-Portilla (2007)
<i>H. rufescens</i>	36	16	12	6	2				Gallardo-Escarate and Del Rio-Portilla (2007)
	36	16	2	16	2				Gallardo-Escarate et al. (2004)
	36	16		18		2			Gallardo-Escarate and Del Rio-Portilla (2007)

^aDiploid chromosome number was estimated from haploid number in meiotic cells; *Ag-NOR number 5 and 6 pairs; **18S-5.8S-28S rDNA located at the terminal region of the long arms of two pairs of SM chromosomes (number 4 and 11 pairs); ***18S-5.8S-28S rDNA located at the terminal region of the long arms of two pairs of SM chromosomes (number 2 and 4 pairs).

Table 2. Summary of triploidization treatments and viability of resultant larvae in abalone based on Okumura (2006).

<i>Haliotis</i> species	Treatment*	Viability of larvae	Reference
<i>H. discus hannai</i>	CS, HS, HPS	Survival 60-90%	Arai et al. (1986)
	CS, CB	Hatch 33-89%	Sun et al. (1993)
	CS	Survival 3-52%	Hasekura (1993)
	HS	Survival 1-64%	Hasekura (1993)
	CB	Survival 12-100%	Hasekura (1993)
	HS+Caffeine	Survival 1-74%	Hasekura (1993)
	Caffeine	Normal 18-75%	Okumura et al. (1996a)
	Caffeine	Normal 31-94%	Okumura et al. (2007)
<i>H. diversicolor diversicolor</i>	CS	Normal 62-74%	Kudo et al. (1991)
<i>H. d. supertexta</i>	CS	Survival 12-16%	Yang et al. (1997)
	6-DMAP	Normal 50%	Yan et al. (2001)
<i>H. d. aquatilis</i>	6-DMAP	Survival 50-55%	Yan and Chen (2002)
<i>H. midae</i>	CB	Survival 12-16%	Stepito and Cook(1998)
<i>H. asinina</i>	6-DMAP	Normal 78-83%	Norris and Preston (2003)
<i>H. rufescens</i>	CB	Survival 1-55%	Maldonado et al. (2001)
<i>H. rubra</i>	6-DMAP, CB	Survival 30-55%	Liu et al. (2004)
<i>H. laevigata</i>	6-DMAP	Survival 17%	Dunstan et al. (2007)
	CB	Hatch 18-57%	Li et al. (2007)

*CS, Cold shock; HS, heat shock; HPS, hydropressure shock; CB, cytochlasin B; 6-DMAP, 6-dimethylaminopurine.

by Miyaki et al. (1995) and they found that survival rate was lower than parental *H. gigantea* throughout the experimental period (341 days), but the growth of hybrid was better than the parental species in the period from the end of October to March. Viable hybrids were also produced by cross-breeding between *H. d. hannai* female and *Haliotis kamtschatkana* male, despite the very low fertilization success (Hoshikawa et al., 1998). The hybrid abalone outperformed in growth than parental species at 18°C, while at low temperature condition, about 8°C hybrids outperformed than *H. d. hannai*, but not *H. kamtschatkana*. The above mentioned studies concluded that some inter-specific hybrid abalone show increased growth than parental species and also suggest that inter-specific hybridization is a potential means to improve growth traits of aquaculture strains.

TRIPLOIDIZATION

There are relatively large numbers of triploid studies in aquatic invertebrates (Elliott 2000; Okumura 2006; Pifferrer et al., 2009) since the first pioneer trial that was done by Arai et al. (1986). Table 2 summarizes triploidization methods and approximate survival of larvae after treatments in abalone examined species. To induce triploidy, the release of the first polar body or the second polar body was inhibited by optimum dose (intensity and duration of treatment) of physical (cold-, heat- or hydrostatic pressure shock) or chemical (cytocharasin B (CB), 6-dimethylaminopurine (6-DMAP), or caffeine) treatments at the optimum starting timing. Precise temperature

regulation apparatus such as “water-bath” is required for temperature treatments (cold or heat shock) of the large quantity of fertilized eggs and very expensive “French Press” is required for hydrostatic pressure treatment. Mass treatment is easier when the fertilized eggs are soaked into the chemical solution. But, CB and 6-DMAP are required to consider the risk-control for environment and workers in hatchery and are very expensive when cost-performance is estimated. For chemical treatment, the caffeine is considered the best because it is a natural product and recognized as safe food-additive and frequently the caffeine treatment achieves 100% triploidization (Okumura et al., 2007). Moreover, it is inexpensive if compared with CB and 6-DMAP as emphasized by Okumura (2006) who calculated that price required for the effective triploidization by caffeine is approximately 1/130 to CB and 1/50 to 6-DMAP treatment.

Generally, due to the side effect of the treatment, high mortalities were recorded in the stages just after the treatment and during larval stages, but later, survival rates become more stable (Okumura 2006). For determination of ploidy status of treated animal, chromosome observation was conducted in the early studies (Arai et al., 1986; Kudo et al., 1991; Okumura, 1991; Okumura et al., 1996a and b), but flow-cytometrical analysis is now a major tool for rapid ploidy determination by automated measurements of nuclear DNA contents of samples (Norris and Preston, 2003; Liu et al., 2004; Li et al., 2007; Okumura et al., 2007). Flow cytometry makes it possible to determine ploidy status of a small piece of tissue sample and individual veliger larva or early juvenile within a relatively short time (Eguchi et al., 2003). Diverse

results have been obtained about gonad development and reproductive capacity of triploid abalone. Hasekura (1993) found the spawning in 4-year-old triploid *H. d. hannai* females and obtained fertilized eggs at high percentages, but they developed abnormally. On the contrary, he did not detect any sperm from triploid males. In small size abalone *H. diversicolor diversicolor*, 2-year-old triploid females had mature oocytes, but the males exhibited no spermatozoa (Kudo et al., 1994). In triploid *H. rubra*, more abnormal gonadal development and gametogenesis were reported (Liu et al., 2009). Triploid females externally lacked an ovary, but they had only a thin layer of oogonia in microscopic level. On the contrary, triploid males showed gonadal development, but the spermatogenesis was arrested at spermatocytes.

Growth is the most attractive trait in abalone farming, because slow growth in abalone is always a serious problem that hinders the sound development of aquaculture. Thus, the estimation of performance has been concentrated to growth in most triploid abalone papers. In *H. d. hannai* and *H. d. discus*, triploid significantly outperform in shell length and body weight than diploid control in the age from 1 to 5-year-old (Hasekura 1993; Sun et al., 1993; Chen et al., 2002). Similar outperformance in growth traits of adult abalones was also reported in *H. diversicolor aquatilis* (Yan and Chen 2002), *H. rubra* (Liu et al., 2004) and *H. laevigata* (Dunstan et al., 2007; Li et al., 2007), while in *H. diversicolor diversicolor*, there were no significant differences in shell length and body weight between triploid and control diploid, but edible part (muscle part) of triploid abalone was significantly larger than that of the control diploid (Kudo et al., 1994). Similar results were obtained in triploid *H. rubra*, in which no significant difference was seen between triploid and diploid, but triploid abalone had a more elongated shell and greater foot muscle (Liu et al., 2009).

Biochemical characteristics have been poorly studied in triploid abalone. Sun et al. (1993) reported higher glycogen content in triploids than diploids. Almost the same amino acid composition between diploid and triploid abalone was reported by Chen et al. (2002). Recently, Dunstan et al. (2007) reported that fatty acid composition of the meat was not affected by ploidy statuses in *H. laevigata*.

There is an argument about the mechanism responsible for better growth in triploid abalone. Outperformance of triploid growth, especially in maturation and spawning season, has been generally explained in fish by the energy reallocation from sexual maturation to somatic growth due to sterility of triploid animals (Piferrer et al., 2009). However, gigantism, such as proportional correlation between ploidy status and body size, which has been found in polyploid plant (Blakeslee, 1941), is likely involved in abalone growth based on examinations on body size (Okumura and Yamamori, 2002), cell size (Harigaya et al., 2001), and cell numbers (Okumura et al., 2002). The involvement of gigantism in better growth is

strongly suggested in bivalve triploids in the dwarf surfclam (*Mulinia lateralis*) (Guo and Allen, 1994) and the Pacific oyster (*Carassostrea gigas*) (Guo et al., 1996).

INDUCED GYNOGENESIS AND TETRAPLOIDIZATION

Gynogenesis is a development without any genetic contribution of paternal genome after triggering the initiation of development by fertilization of eggs with genetically inactivated sperm. Gynogenesis is considered an important manipulation technique to produce monosex populations as well as inbred lines. Arai et al. (1984) induced the gynogenesis with UV-irradiated sperm in *H. d. hannai* for the first time, but resultant progeny were inviable haploid larvae. Induction of gynogenetic development is generally difficult in shellfish due to the susceptibility of spermatozoa to UV irradiation (Li et al., 2000a, b), but a few studies reported the production of gynogenetic diploid abalone by inhibition of the polar body release after initiation of haploid development (Fujino et al., 1990b; Cai et al., 2004; Li et al., 1999; Li and Kijima, 2005; Nie et al., 2011).

Tetraploid abalone is important as the source of diploid gametes because mass production of triploid can be simply realized by cross breeding between diploid (haploid gametes) and induced tetraploid (diploid gametes). Tetraploid abalone can be theoretically induced by the possible occurrence of endomitosis after inhibition of the first cleavage, but no viable progeny has been produced by this method (Zhang et al., 2000). Alternatively, Okumura et al. (1998a, b) reported the production of tetraploid abalone larvae by inhibition of both the first and the second polar body release with CB and caffeine, respectively, after the initiation of gynogenetic haploid development by fertilization of eggs with UV-irradiated sperm. Inhibition of both meiotic divisions by cold shock was also examined (Okumura et al., 1996b). However, no viable tetraploid progeny has been produced.

GENETIC MAP AND RELATED QTL ANALYSES FOR MARKER-ASSISTED SELECTION

Genetic mapping is important not only for quantitative trait loci (QTL) analysis to elaborate aquaculture technology (Yu and Guo, 2006), but also for comparative synteny of genomes to get insights into evolutionary process of the target species (Barbazuk et al., 2000; Naruse et al., 2004). Since relatively large numbers of polymorphic microsatellite markers were successfully developed in *H. d. hannai* (Sekino and Hara, 2001, 2007; Sekino et al., 2005, 2006). The first linkage mapping for the abalone was conducted based on 180 microsatellite DNA markers (Sekino and Hara, 2007b). The numbers of linkage groups in the female and male map were 19 and 18, respectively, and almost or just same to the haploid chromosome number ($n=18$). As in linkage maps of other marine animals and fishes, female map (about 900 cM) is much

longer than male map (about 700 cM), suggesting higher recombination rates in females than males. The maps are useful to identify gene(s) related to or influenced on the commercially attractive traits such as growth, survival, disease resistance and environment adaptability. Recently, linkage map was also constructed by using 308 micro-satellite markers in *H. diversicolor* (Zhan et al., 2012). The number of linkage groups was consistent with the haploid chromosome number ($n=16$) and female map (about 760 cM) was longer than male map (about 680 cM) as observed in the map of *H. discus hannai* (Sekino and Hara, 2007b).

Gene (marker)-centromere map was reported for the allozyme loci by half-tetrad analysis in triploid *H. d. hannai* (Fujino et al., 1987; 1988a, b, 1989, 1990a) and in triploid *H. diversicolor diversicolor* (Fujino et al., 1991, 1992). By such approaches, allozyme loci were mapped in relation to the centromere of chromosomes. Half-tetrad analysis using polymorphic molecular markers and gynogenetic diploid families successfully created much more precise gene(marker)-centromere map in *H. d. hannai* (Li and Kijima, 2005; Nie et al., 2011).

CRYOPRESERVATION

Cryopreservation of germ cells, gametes (egg and sperm) and zygotes (fertilized eggs, embryos, larvae) is very important from the view point of preservation of biodiversity as well as efficacy improvement in aquaculture production. According to Tsai and Chao (1994), abalone sperm can be successfully cryopreserved for long time and they maintain high fertilizability about one year after storage and thawing. Such a successful result in cryopreservation of abalone sperm can make it possible to hybridize two different species that are distributed in geographically different places and that reproduce in different seasons. However, at present, no studies have been done on hybridization using cryopreserved sperm. Preservation of eggs and embryos has not been reported yet.

TRANSGENICS

Transgenic technology is necessary to study its future potential for genetic improvement as well as its application to expand our basic information on genes, genetics and genomics of aquatic organisms. At present, however, there are many arguments on public concerns about the use of genetically modified (GM) fish and shellfish from the viewpoint of food safety and security as well as environment safety including bio-diversity preservation. Gene transfer into abalone was tried by the electroporation of sperm in *Haliotis iris* by Sin et al. (1995) and then in *H. diversicolor supertexta* by Tsai et al. (1997). Gene transfer by the electroporation of embryos was also reported in *H. rufescens* by Powers et al. (1995). As mentioned above, transgenic in abalone was done by

electroporation and no micro-injection was reported presumably due to difficulties of the very small size of eggs.

CONCLUSION

The present review paper concludes that both hybridization and triploidization are useful to improve growth traits in abalone. Thus, the combination of both methods, such as production of hybrid triploid strain (allo-triploidization) is potentially effective to improve growth. Although such approach is considered promising as a farm strain, their production has not been attempted yet. For further progress on chromosome manipulation studies in abalone, experimental hybridization between remotely related abalone species, for example between the abalone with $2n=32$ chromosomes and those with $2n=36$ chromosomes, are interesting. The successful sperm cryopreservation technique may enable us to cross-breed between the two species which are distributed in geographically different regions or presents reproductive asynchrony. Allo-triploidization between the remotely related abalone species is very interesting, because resultant allo-triploidy is likely to disrupt normal meiosis and gametogenesis and then increase the probability of sterilization than diploid hybrid and auto-triploid situations, due to the presence of one additional heterospecific genome (chromosome set).

Construction of much more precise genetic map will promote QTL analyses and then realize marker-assisted selection (MAS) to form aquaculture strains in abalone. However, the present genetic linkage map is still primitive because of the low coverage of whole genome. Further mapping using more co-dominant DNA markers is necessary to construct more detailed map for successful selection and gene identification. In addition, quantitative genetic studies are also required for abalone selection program. Although studies on quantitative traits have been done by many groups (Hara, 1990; Hara and Kikuchi, 1992; Kobayashi and Fujio, 1994, 1996; Kawahara et al. 1997), results are still fragmentary and inconclusive.

In conclusion, strategic studies on genetic improvement programs are necessary in aquaculture abalone strains by using classical genetic as well as modern biotechnological approaches. Further, genetic and genomic studies are now necessary to deepen our knowledge and then to expand it to application.

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REFERENCES

- Adachi K, Okumura S (2012). Determination of genome size of *Haliotis discus hannai* and *H. diversicolor aquatilis* (Haliotidae) and phylogenetic examination of this family. *Fish. Sci.* 78: 849-852.
- Ahmed F, Koike Y, Strussmann CA, Yamasaki I, Yokota M, Watanabe S (2008). Genetic characterization and gonad development of artificially produced interspecific hybrids of the abalones, *Haliotis discus discus* Reeve, *Haliotis gigantea* Gmelin and *Haliotis madaka* Habe. *Aqua. Res.* 39: 532-541.
- Amar-Basulto G, Lafarga-De La Cruz F, Iturra-Constant P, Gallardo-Escarate C (2011). Karyotype analysis of interspecific hybrids between *Haliotis rufescens* and *Haliotis discus hannai*. *Aquac. Res.* 42: 1460-1466.
- An HS, Jee YJ, Min BL, Han SJ (2005). Phylogenetic analysis of six species of pacific abalone (Haliotidae) based on DNA sequences of 16S rRNA and cytochrome c oxidase subunit I mitochondrial gene. *Mar. Biotechnol.* 7: 373-380.
- Arai K, Wilkins NP (1986). Chromosomes of *Haliotis tuberculata* L. *Aquac.* 58: 305-308.
- Arai K, Tsubaki H, Ishitani Y, Fujino K (1982). Chromosomes of *Haliotis discus hannai* Ino and *H. discus* Reeve. *Bull. Japan. Soc. Sci. Fish.* 48: 1689-1691.
- Arai K, Naito F, Sasaki H, Fujino K (1984). Gynogenesis with ultraviolet ray irradiated sperm in the Pacific abalone. *Bull. Japan. Soc. Sci. Fish.* 50: 2019-2023.
- Arai K, Naito F, Fujino K (1986). Triploidization of the Pacific abalone with temperature and pressure treatments. *Bull. Japan. Soc. Sci. Fish.* 52: 417-422.
- Arai K, Fujino K, Kudo M (1988). Karyotype and zymogram differences among three species of the abalone *Haliotis planata*, *H. varia* and *H. diversicolor diversicolor*. *Nippon Suisan Gakkaishi* 54:2055-2064.
- Barbazuk WB, Korf I, Kadavi C, Heyen J, Tate S, Wun E, Bedell JA, Mcpherson JD, Johnson SL (2000). The syntenic relationship of the zebrafish and human genomes. *Genome Res.* 10: 1351-1358.
- Blakeslee AF (1941). Effect of induced polyploidy in plants. *Am. Nat.* 75: 117-135.
- Cai M, Ke C, Wang Z, Zhou S, Zhang Z, Wang Y, Zhang Z (2004). Induction of gynogenetic diploid in the Pacific abalone, *Haliotis diversicolor supertexta*. *J. Shellfish Res.* 23: 1115-1121.
- Chen Q, Yang J, Gao A, Hu X, Zhao K, Zhao J (2002). A study on the growth comparisons of triploid of *Haliotis discus* Reeve. *Donghai Mar. Sci.* 20: 19-54.
- Colombera D, Tagliaferri F (1983). Chromosomes from male gonads of *Haliotis tuberculata* and *Haliotis lamellosa* (Haliotidae, Archo-gasteropoda, Mollusca). *Caryologia* 36: 231-234.
- Dunstan GA, Elliott NG, Appleyard SA, Holmes BH, Conod N, Grubert MA, Cozens MA (2007). Culture of triploid greenlip abalone (*Haliotis laevigata* Donovan) to market size: Commercial implications. *Aquaculture* 271: 130-141.
- Eguchi H, Okumura S, Arai K, Sakai M, Nagahara T, Norimatsu M, Furukawa S, Yamamori K (2003). Pretreatment method for flow cytometry of larval and early juvenile Pacific abalone *Haliotis discus hannai* using the ultrasonic generator. *Fish Genet. Breed. Sci. (Suisan Ikusyu)* 33: 35-39 (In Japanese with English abstract).
- Elliott NG (2000). Genetic improvement programmes in abalone: what is the future. *Aquac. Res.* 13: 51-59.
- FAO (2010). The State of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department, Rome, 218 p.
- Franchini P, Slabbert R, Van der Merwe M, rous A, Roodt-Wilding R (2010) Karyotype and genome size estimation of *Haliotis midae*: estimators to assist future studies on the evolutionary history of Haliotidae. *J. Shellfish Res.* 29: 941-950.
- Fujino K, Kamakura M, Mito R, Arai K (1987). Overdominance of survivals at phosphoglucomatase dehydrogenase locus in triploid Pacific abalone. *Nippon Suisan Gakkaishi* 53: 1759-1764.
- Fujino K, Nakajima S, Sawada H (1988a). Differential contribution of oocytes to triploid offsprings due to maternal genotypes at phosphoglucomatase thermostability variation locus in the Pacific abalone. *Nippon Suisan Gakkaishi* 54: 953-958.
- Fujino K, Nakajima S, Takahashi T (1988b). Differential contribution of oocytes to triploid offsprings due to maternal genotypes at multiple loci in the Pacific abalone. *Nippon Suisan Gakkaishi* 54: 2049-2054.
- Fujino K, Kaneko R, Ikeda T, Arai K, Okumura S (1989). Estimating the rate of gene-centromere recombination at leucine aminopeptidase, malic enzyme and phosphoglucomatase loci in the Pacific abalone. *Nippon Suisan Gakkaishi* 55: 1875-1883.
- Fujino K, Nakajima S, Ogasawara Y, Yonezawa Y, Arai K, Okumura S (1990a). Estimating rate of gene-centromere recombination at alpha-glycerophosphate dehydrogenase, superoxide dismutase, and esterase loci in the Pacific abalone. *Nippon Suisan Gakkaishi* 56: 1371-1381.
- Fujino K, Arai K, Iwadare K, Yoshida T, Nakajima S (1990b). Induction of gynogenetic diploid by inhibiting second meiosis in the Pacific abalone. *Nippon Suisan Gakkaishi* 56: 1755-1763.
- Fujino K, Arai K, Kudo M, Suzuki T, Masumori M, Takahashi K (1991). Estimating rate of gene-centromere recombination at some isozyme loci in *Haliotis diversicolor diversicolor*. *Nippon Suisan Gakkaishi* 57: 1477-1485.
- Fujino K, Arai K, Kudo Takahashi K, Uematsu T, Okumura S (1992). Estimating rate of gene-centromere recombination at some isozyme loci in *Haliotis diversicolor diversicolor* and *H. discus hannai*. *Nippon Suisan Gakkaishi* 58: 445-459.
- Gallardo-Escarate C, Alvarez-Borrego J, Del Rio-Portilla MA, Kober V (2004). Karyotype of Pacific red abalone *Haliotis rufescens* (Archoegastropoda: Haliotidae) using image analysis. *J. Shellfish Res.* 23: 205-209.
- Gallardo-Escarate C, Alvarez-Borrego J, Del Rio-Portilla MA, Von Brand-Skopnik E, Cross I, Merlo A, Rebordino L (2005). Karyotype analysis and chromosomal localization by FISH of ribosomal DNA, telomeric (TTAGGG)_N and (GATA)_N repeats in *Haliotis fulgens* and *H. corrugata* (Archoegastropoda: Haliotidae). *J. Shellfish Res.* 24: 1153-1159.
- Gallardo-Escarate C, Del Rio-Portilla MA (2007). Karyotype composition in three California abalones and their relationship with genome size. *J. Shellfish Res.* 26: 825-832.
- Guo X, Allen Jr SK (1994). Sex determination and polyploid gigantism in the dwarf surfclam (*Mulinia lateralis* Say). *Genetics* 138: 1199-1206.
- Guo X, DeBrosse GA, Allen Jr SK (1996). All-triploid Pacific oyster (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploid. *Aquaculture* 142: 149-161.
- Hara M (1990). The effect of genetics on growth in three groups of abalone seeds. *Bull. Tohoku Natl Fish. Res. Instit.* 52:73-77 (In Japanese).
- Hara M (2008a). Current status on genetics and breeding studies of abalone in Japan. *Fish. Genet. Breed. Sci. (Suisan-Ikusyu)* 38: 31-39 (In Japanese).
- Hara M (2008b). Genetic management for wild populations of abalone. *J Anim. Genet.* 36:105-115 (In Japanese).
- Hara M, Kikuchi S (1992). Increasing the growth rate of abalone, *Haliotis discus hannai*, using selection techniques. NOAA Technical Report NMFS 106: 21-26.
- Hara M, Sekino M (2005). Genetic difference between Ezo-awabi *Haliotis discus hannai* and Kuro-awabi *H. discus discus* populations: Microsatellite-based population analysis in Japanese abalone. *Fish. Sci.* 71: 754-766.
- Harigaya Y, Okumura S, Ozawa M, Hisatsune T, Furukawa S, Yamamori K (2001). Cell size and cell number in triploid larvae of the Pacific abalone, *Haliotis discus hannai*. *Fish. Genet. Breed. Sci. (Suisan-Ikusyu)* 31: 51-55 (In Japanese with English abstract).
- Hasekura O (1993). Baisutai ni yoru idennheni no yuhatsu gijyutsu, awabi, kaki tou no ikusyu gijyutu no kaihatu (Induction technology of genetic variation by polyploidy, development of breeding technology in abalone, oyster and others). *Agric., For. Fish. Res. Council*, pp.78-90 (In Japanese).
- Hernandez-Ibarra N, Marquez C, Ramirez JL, Ibarra AM (2004) Comparative karyotypes of two northeastern Pacific abalone species (*Haliotis fulgens* Philippi and *Haliotis rufescens* Swainson). *J. Shellfish Res.* 23: 861-865.
- Hoshikawa H, Sakai Y, Kijima A (1998). Growth characteristics of the hybrid between pinto abalone *Haliotis kamtschatkana* Jones, and ezo abalone, *H. discus hannai* Ino, under high and low temperature. *J. Shellfish Res.* 17: 673-677.
- Ibarra AM, Hernandez-Ibarra NK, Cruz P, Perez-Enriquez R, Avila S,

- Ramirez JL (2005). Genetic certification of presumed hybrids of blue x red abalone (*Haliotis fulgens* Philippi and *H. rufescens* Swainson). *Aquac. Res.* 36: 1356-1368.
- Jarayabhand P, Paphavasin N (1996). A review of the culture of tropical abalone with special reference to Thailand. *Aquac.* 140: 159-168.
- Jarayabhand P, Yom-La R, Popongviwat A (1998). Karyotypes of marine mollusks in the family Haliotidae found in Thailand. *J. Shellfish Res.* 17: 761-764.
- Kawahara I, Noro T, Omori M, Hasekura O, Kijima A (1997). Genetic progress for Growth in different selected populations of abalone, *Haliotis discus hannai*, at different hatcheries. *Fish. Genet. Breed. Sci. (Suisan-Ikusu)* 25: 81-90 (In Japanese).
- Kobayashi M, Fujio Y (1994). Genetic study on metric traits of the Pacific abalone *Haliotis discus hannai*. *Tohoku J. Agricul. Res.* 44: 59-66.
- Kobayashi M, Fujio Y (1996). Genetic study on shell shape and growth-related traits in the Pacific abalone *Haliotis discus hannai*. *Tohoku J. Agricul. Res.* 46: 141-147.
- Koike Y, Sun ZX, Takashima F (1988). On the feeding and growth of juvenile hybrid abalone. *Suisanzoshoku (Aquaculture Sci.)* 36: 231-235 (In Japanese with English abstract).
- Kudo M, Arai K, Fujino K (1991). Triploidization of *Haliotis diversicolor diversicolor* by cold shock. *Nippon Suisan Gakkaishi* 57: 1263-1267.
- Kudo M, Arai K, Kimoto T, Minagawa M, Fujino K (1994). Survival, growth, and maturation of triploid abalone, *Haliotis diversicolor diversicolor*. *Suisanzoshoku (Aquaculture Sci.)* 42: 605-613 (In Japanese with English abstract).
- Lee Y, Vacquiere VD (1995). Evolution and systematic in Haliotidae (Mollusca: Gastropoda): Inferences from DNA sequences of sperm lysine. *Mar. Biol.* 124: 267-278.
- Li Q, Kijima A (2005). Segregation of microsatellite alleles in gynogenetic diploid Pacific abalone (*Haliotis discus hannai*). *Mar. Biotechnol.* 7: 669-676.
- Li Q, Osada M, Kashihara M, Hirohashi K, Kijima A (1999). Artificially induced gynogenetic diploid in the Pacific abalone, *Haliotis discus hannai* Ino. *Fish. Genet. Breed. Sci.* 28: 85-94.
- Li Q, Osada M, Kashihara M, Hirohashi K, Kijima A (2000a). Cytological observations on nuclear behavior in normal and gynogenetic eggs of the Pacific oyster *Crassostrea gigas*. *Suisanzoshoku (Aquaculture Sci.)* 48: 193-198.
- Li Q, Osada M, Kashihara M, Hirohashi K, Kijima A (2000b). Cytological studies on artificially induced gynogenesis in the Pacific abalone. *Fish. Sci.* 66: 701-707.
- Li Q, Park C, Kijima A (2002). Isolation and characterization of microsatellite loci in the Pacific abalone, *H. discus hannai*. *J. Shellfish Res.* 21: 811-815.
- Li Y, Li X, Qin JG (2007). Triploidy induction in Australian greenlip abalone *Haliotis laevigata* (Donovan) with cytochalasin B. *Aquac. Res.* 38: 487-492.
- Liu W, Heasman M, Simpson R (2004). Induction and evaluation of triploidy in the Australian blacklip abalone, *Haliotis rubra*: a preliminary study. *Aquaculture* 233: 79-92.
- Liu W, Heasman M, Simpson R (2009). Growth and reproductive performance of triploid and diploid blacklip abalone, *Haliotis rubra* (Leach, 1814). *Aquac. Res.* 40: 188-203.
- Luo X, Ke C-H, You W-W, Wang D-X, Chen F (2010). Molecular identification of interspecific hybrids between *Haliotis discus hannai* Ino and *Haliotis gigantea* Gmelin using amplified fragment-length polymorphism and microsatellite markers. *Aquac. Res.* 41: 1827-1834.
- Maldonado R, Ibara RAM, Ramirez JL, Avila S, Vazquez JE, Badillo LM (2001). Induction of triploidy in Pacific red abalone (*Haliotis rufescens*). *J. Shellfish Res.* 20: 1071-1075.
- Minkler J (1977). Chromosomes of the black abalone (*Haliotis cracherodii*). *Specialia* 15: 1143.
- Miyaki K, Niiyama H, Tabeta O (1995). Growth and survival of artificial hybrid abalone, *Nordotis gigantea*♀ x *N. discus*♂ under laboratory conditions. *Suisanzoshoku (Aquaculture Sci.)* 43: 401-405 (In Japanese with English abstract).
- Miyaki K, Tabeta O, Kayano H (1997). Karyotypes of the two species of abalone *Nordotis discus* and *N. gigantea*. *Fish. Sci.* 63: 179-180.
- Miyaki K, Matsuda M, Tabeta O (1999). Karyotype of the giant abalone, *Nordotis madaka*. *Fish. Sci.* 65: 317-318.
- Naganuma T, Hisadome K, Shiraiishi K, Kojima H (1998). Molecular distinction of two resemblant abalone, *Haliotis discus discus* and *Haliotis discus hannai* by 18SrDNA sequences. *J. Mar. Biotech.* 6: 59-61.
- Nakamura HK (1985). The chromosomes of *Haliotis diversicolor aquatilis* (Archaeogastropoda: Haliotidae). *Mar. Rev.* 18: 113-114.
- Nakamura HK (1986). Chromosomes of Archaeogastropoda (Mollusca: Prosobranchia), with some remarks on their cytotaxonomy and phylogeny. *Publ. Seto Mar. Biol. Lab.* 31: 191-267.
- Naruse K, Tanaka M, Mita K, Shima A, Postlethwait J, Mitani H (2004) A medaka gene map: The trace of ancestral vertebrate proto-chromosomes revealed by comparative gene mapping. *Genome Res.* 14: 820-828.
- Nie H, Li Q, Kong L (2011). Centromere mapping in the Pacific abalone (*Haliotis discus hannai*) through half-tetrad analysis in gynogenetic diploid families. *Anim. Genet.* 43: 290-297.
- Norris BJ, Preston NP (2003). Triploid induction in the tropical abalone, *Haliotis asinina* (Linne), with 6-dimethylaminopurine. *Aquac. Res.* 34: 261-264.
- Oakes FR, Ponte RD (1996). The abalone market: opportunities for cultured abalone. *Aquaculture* 140: 187-195.
- Okumura S (1991). A biopsy method for obtaining chromosome preparation from epipoidal tentacle tissue of the Pacific abalone. *Nippon Suisan Gakkaishi* 57: 2337.
- Okumura S (2006). Studies on chromosome manipulation and breeding in abalone. *Fish. Genet. Breed. Sci. (Suisan Ikusu)* 35: 157-163 (In Japanese with English abstract).
- Okumura S, Yamamori K (2002). Large larvae of Pacific abalone, *Haliotis discus hannai* obtained from triploid batches. *Aquac.* 204: 236.
- Okumura S, Furukawa S, Sugie T, Sekimiya D, Toda A, Yamamori K (1996a). Triploid larvae of pacific abalone *Haliotis discus hannai* (Archaeogastropoda: Haliotidae) induced by suppression of meiotic division using caffeine treatment. *Chromo. Info. Serv.* 61: 10-12.
- Okumura S, Furukawa S, Terada E, Yamaguchi Y, Yamamori K (1996b). Gynogenetic tetraploid larvae of the Pacific abalone *Haliotis discus hannai* induced by suppression of the 1st and 2nd meiotic division. *Fish. Genet. Breed. Sci. (Suisan Ikusu)* 23: 21-27. (In Japanese with English abstract).
- Okumura S, Furukawa S, Sasaki T, Shimaoka M, Horikawa C, Yamamori K (1998a). Induction of tetraploid larvae in the Pacific abalone *Haliotis discus hannai* by suppression of the meiotic division using cytocharasin B. *Fish Genet. Breed. Sci. (Suisan Ikusu)* 26: 49-53 (In Japanese with English abstract).
- Okumura S, Furukawa S, Sasaki T, Shimaoka M, Horikawa C, Yamamori K (1998b). Suppression of both 1st and 2nd meiotic divisions by caffeine treatment for the production of tetraploid Pacific abalone, *Haliotis discus hannai* (Archaeogastropoda: Haliotidae). *Chromo. Sci.* 2: 123-127.
- Okumura S, Kinugawa S, Fujimaki A, Kawai W, Maehata H, Yoshioka K, Yoneda R, Yamamori K (1999). Analysis of karyotype, chromosome banding, and nucleolus organizer region of Pacific abalone, *Haliotis discus hannai* (Archaeogastropoda: Haliotidae). *J. Shellfish Res.* 18: 605-609.
- Okumura S, Eguchi H, Harigaya Y, Nakaoka K, Furukawa S, Yamamori K (2002). Cell number in diploid and triploid veliger larvae of Pacific abalone *Haliotis discus hannai*. *Fish. Genet. Breed. Sci.* 32: 103-107.
- Okumura S, Arai K, Harigaya Y, Eguchi H, Sakai M, Senbokuya H, Furukawa S, Yamamori K (2007). Highly efficient induction of triploid Pacific abalone *Haliotis discus hannai* by caffeine treatment. *Fish. Sci.* 73: 237-243.
- Pifferrer F, Beaumont A, Falguiere JC, Flajshans M, Haffray P, Colombo L (2009). Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture* 293: 125-156.
- Powers DA, Kirby VL, Cole T, Hereford L (1995). Electroporation as an effective means to introducing DNA into abalone (*Haliotis rufescens*) embryos. *Mol. Mar. Biol. Biotech.* 4: 369-375.
- Sekino M, Hara M (2001). Microsatellite DNA loci in Pacific abalone *Haliotis discus discus* (Mollusca, Gastropoda, Haliotidae). *Mol. Ecol.*

- Notes, 1: 8-10.
- Sekino M, Hara M (2007a). Individual assignment tests proved genetic boundaries in a species complex of Pacific abalone (genus *Haliotis*). *Coserv. Genet.* 8: 823-841.
- Sekino M, Hara M (2007b). Linkage maps for the Pacific abalone (genus *Haliotis*) based on microsatellite DNA markers. *Genetics* 175: 945-958.
- Sekino M, Saido T, Fujita T, Kobayashi T, Takami H (2005). Microsatellite DNA markers of Ezo abalone (*Haliotis discus hannai*): a preliminary assessment of natural populations sampled from heavily stocked areas. *Aquaculture* 243: 453-466.
- Sekino M, Kobayashi T, Hara M (2006). Segregation and linkage analysis of 75 novel microsatellite DNA markers in pair crosses of Japanese abalone (*Haliotis discus hannai*) using the 5'-tailed primer method. *Mar. Biotechnol.* 8: 453-466.
- Sin FYT, Mukherjee UK, McKenzie JC, Sin IL (1995). Electroporation of abalone sperm enhances sperm-DNA association. *J. Fish Biol.* 47 (supple. A): 20-28.
- Stepto NK, Cook PA (1998). Induction of triploidy in the South African abalone using cytochalasin B. *Aquac. Int.* 6: 161-169.
- Streit K, Geiger DL, Lieb B (2006). Molecular phylogeny and the geographic origin of Haliotidae traced by haemocyanin sequences. *J. Molluscan Stud.* 72: 105-110.
- Sun Z, Li N, Song Z, Zhao Y, Guan X (1993). The condition of triploid induction in abalone *Haliotis discus hannai* and its indoor raised experiment. *J. Fish. China* 17: 243-248 (In Chinese).
- Tsai HP, Chao NH (1994). Cryopreservation of small abalone (*Haliotis diversicolor*) sperm-technique and its significance. *J. Fish. Soc. Taiwan* 21: 347-360.
- Tsai HJ, Lai CH, Yang HS (1997). Sperm as a carrier to introduce an exogenous DNA fragment into the oocyte of Japanese abalone (*Haliotis diversicolor supertexta*). *Transgenic Res.* 6: 85-95.
- Van der Merwe M, Rdoody-Wilding R (2008) Chromosome number of the south African abalone *Haliotis midae*. *Afr. J. Mar. Sci.* 30: 195-198.
- Wang Q, Ma Q, Wang Y (1988) The karyotype study of *Haliotis discus hannai* Ino. *Zool. Res.* 9: 171-174.
- Yan Z, Chen J (2002). Seed breeding and culturing of triploid abalone *Haliotis diversicolor aquatilis*. *J. Fish. China* 26: 54-60 (In Chinese with English abstract).
- Yan Z, Chen J, Wu P, Gao X (2001). Triploid of the abalone *Haliotis diversicolor supertexta* induced with 6-DMAP: by blocking the second polar body of the zygotes. *J. Oceanogr. Taiwan Strait* 20: 15-19 (In Chinese with English abstract).
- Yang HS, Ting YY, Chen HC (1997). Effect of cold shock on the production of triploid zygotes and the embryonic development of small abalone, *Haliotis diversicolor supertexta* Lischke. *Acta. Zool. Taiwan* 8: 67-78.
- Yu Z, Guo X (2006) Identification and mapping of disease-resistance QTLs in the eastern oyster, *Crassostrea virginica* Gmelin. *Aquaculture* 254: 160-170.
- Zhan X, Fan F, You W, Yu J, Ke C (2012) Construction of an integrated map of *Haliotis diversicolor* using microsatellite markers. *Mar. Biotechnol.* 14: 79-86.
- Zhang G, Wang Z, Chang Y, Song J, Ding J, Zhao S, Guo X (2000). Tetraploid induction in the pacific abalone *Haliotis discus hannai* Ino with 6-DMAP and CB. *J. Shellfish Res.* 19: 540-541.