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Population genetics of the Pacific abalone (*Haliotis discus hannai*) in Korea inferred from microsatellite marker analysis

H.S. An, J.W. Lee and J.Y. Park

Biotechnology Research Division,
National Fisheries Research and Development Institute, Busan, Korea

Corresponding author: H.S. An
E-mail: hsan@nfrdi.go.kr

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ABSTRACT. Populations of the Pacific abalone, *Haliotis discus hannai*, have been severely overexploited over the past few decades in Korea. Information regarding the levels of genetic variability and structure within populations is insufficient for the development of effective strategies for conservation of genetic diversity of this species. To assess the genetic status of this species, we examined variation at six microsatellite loci in 842 individuals of Pacific abalone collected from three hatchery stocks of the main aquaculture areas and eight wild populations, which were two from the East Sea, two from the West Sea and three from the South Sea. High levels of polymorphism at these microsatellite loci were found in both the wild and hatchery populations. The genetic variation in the hatchery stocks [overall number of alleles (N_A) = 24.00; allelic richness (A_R) = 19.71; observed heterozygosity (H_O) = 0.733] was similar to that of the wild (overall N_A = 28.13; A_R = 22.62; H_O = 0.775) populations. Low levels of inbreeding and significant Hardy-Weinberg equilibrium deviations were detected in both the wild and hatchery populations. Significant F_{ST} values were observed for the hatchery stocks and in most cases between the wild and hatchery populations (overall F_{ST} = 0.017, P < 0.01); however, only a

minor portion of the genetic diversity was distributed between the wild and hatchery populations. These results reflect intensive seedling and stocking practices. This preliminary study showed genetic separation between the eastern and pooled western and southern wild populations in Korea, which was based on the F_{ST} value, phylogenetic tree clustering, PCA and MDS analyses, structure analysis, and AMOVA. This strong biogeographic structure of *H. d. hannai* in Korea may be considered to be independent management units. This study demonstrates the feasibility of microsatellite analyses for the monitoring of genetic diversity and for revealing the population structure of the wild Pacific abalone. This information will be useful for the proper management and conservation of *H. d. hannai* in Korea.

Key words: *Haliotis discus hannai*; Pacific abalone; Microsatellites; Genetic diversity; Genetic structure

INTRODUCTION

The Pacific abalone *Haliotis discus hannai*, which is referred to as Cham-jeonbok, is an important aquaculture species that has been cultured in Korea for the past three decades. Thus, artificially propagated breeding programs and culture techniques for *H. d. hannai* have been well developed. In contrast to the rapid growth of the aquacultural population, natural populations of Pacific abalone are believed to be declining in their native regions as a result of habitat destruction and over-fishing, as with many other marine fishery species around the world (Hilborn et al., 2003). The recent annual landing of this species has been reduced to less than half of the volume (approximately 70 tons in 2008) (Fisheries Information Service, 2009) of the average catch level (116-197 tons since 2005) (Fisheries Information Service, 2009). To alleviate fishing pressures on the wild populations, the aquacultural abalone population has increased, and enhancement practices have been intensively continued to release hatchery stock into the natural coastal areas across Korea every year. At present, the annual production of cultured *H. d. hannai* has increased nearly 40-fold (approximately 7000 tons) when compared with the annual landing of this species in 2011 (Fisheries Information Service, 2009). Thus, the genetic impact of stocked hatchery abalone on natural resources is a growing concern to those attempting to maintain a sustainable fishery, because hatchery stocks may exhibit genetic changes that threaten the integrity of the wild populations by eliminating genetic variation from the gene pool (Hurt and Philip, 2004). Therefore, monitoring the genetic differences between hatchery stocks and wild populations is recommended to preserve genetic variation in the natural populations (FAO, 1993). Furthermore, in recent years, the production and economic success of this species has been hindered by factors, such as slow juvenile growth rates, low larval survival rates and increased juvenile mortality due to high water temperatures. These impediments have fostered the need for a basic understanding of the genetic characteristics of the population in relation to practical management. However, despite the importance of Pacific abalone for commercial aquaculture in Korea, little is known about its genetic background and the genetic differences between cultured stocks and wild populations or between geographical populations. Therefore, it is necessary to elucidate the genetic structure of Pacific abalone in Korea.

Understanding the processes and organization of genetic diversity and genetic resources is essential for the sustainable management of exploited fishery populations. Thus, obtaining knowledge regarding patterns of stock structure is a prerequisite for successful aquaculture management and the preservation of aquatic biodiversity in the sustainable development of marine fisheries (Reiss et al., 2009). Elucidating the present population structure, levels of gene flow, and genetic diversity within and between populations that are harvested for commercial purposes can provide useful information for the development of appropriate conservation and management strategies. With the rapid development of abalone aquaculture and breeding projects, molecular markers for studying the genetic variation among wild populations may aid the elucidation of the genetic differences among wild populations, assess the genetic variation within hatchery stocks and determine the genetic impacts of aquaculture on wild populations, thereby promoting sustainable aquaculture.

Many molecular markers are available for use in studying the population genetics of marine fishery species, including allozyme, mitochondrial DNA and microsatellite markers. Among them, microsatellite markers have been widely used for studies of genetic variation because of their high levels of polymorphism and relative ease of development and use (Liu et al., 2009).

Several studies have assessed the development of a variety of microsatellite markers in Pacific abalone (Sekino and Hara, 2001; Li et al., 2002; Sekino et al., 2005; An et al., 2010a,b), and several population genetic studies using microsatellite loci have reported significant differences of genetic diversity between wild and hatchery abalone stocks in Japan and China (Li et al., 2004, 2007; Hara and Sekino, 2007). In Korea, there are two reports regarding the genetic characterization of new microsatellite markers from Pacific abalone (An et al., 2010a,b) and only one report on the genetic diversity of hatchery stocks, which found high levels of genetic variation and highly significant F_{ST} values among five hatchery-cultured Pacific abalone populations in Korea (An et al., 2011b).

In the current study, the genetic diversity and structure of wild populations of *H. d. hannai* that were collected from eight different regions in Korea were analyzed based on six microsatellite markers. In addition, the genetic variation of three hatchery populations was compared with that of the wild populations. This study will provide useful data for the genetic conservation and management of wild abalone populations and for the production of high-quality abalone through selective breeding programs.

MATERIAL AND METHODS

Sample collection and DNA extraction

To sample the main natural habitats in Korea, a total of 842 Pacific abalones (*H. d. hannai*) were collected from eight locations around the Korean Peninsula and three hatcheries that were located on the western and southern coasts, which are the main aquaculture areas of Pacific abalones. The sampling locations are shown in Figure 1. All of the samples were obtained between October 2003 and September 2004 by direct sampling (Table 1). For the three hatchery-reared populations (BOH, YSH and JJH), the original ancestors of the hatchery abalone were collected locally and the hatchery populations had been reared

continuously, but information regarding the exact origins of individuals from each broodstock and the management practices that were applied to the cultured stocks was not available. Two wild populations, SCN (N = 83) and PHN (N = 82), were collected from the East Sea near Sokcho and Pohang, respectively. From the West Sea, two additional wild populations, BRN (N = 71) and BAN (N = 87), were collected. Another four populations, WDN (N = 96), YSN (N = 90), CJN (N = 70), and JJN (N = 37), were collected from the South Sea.

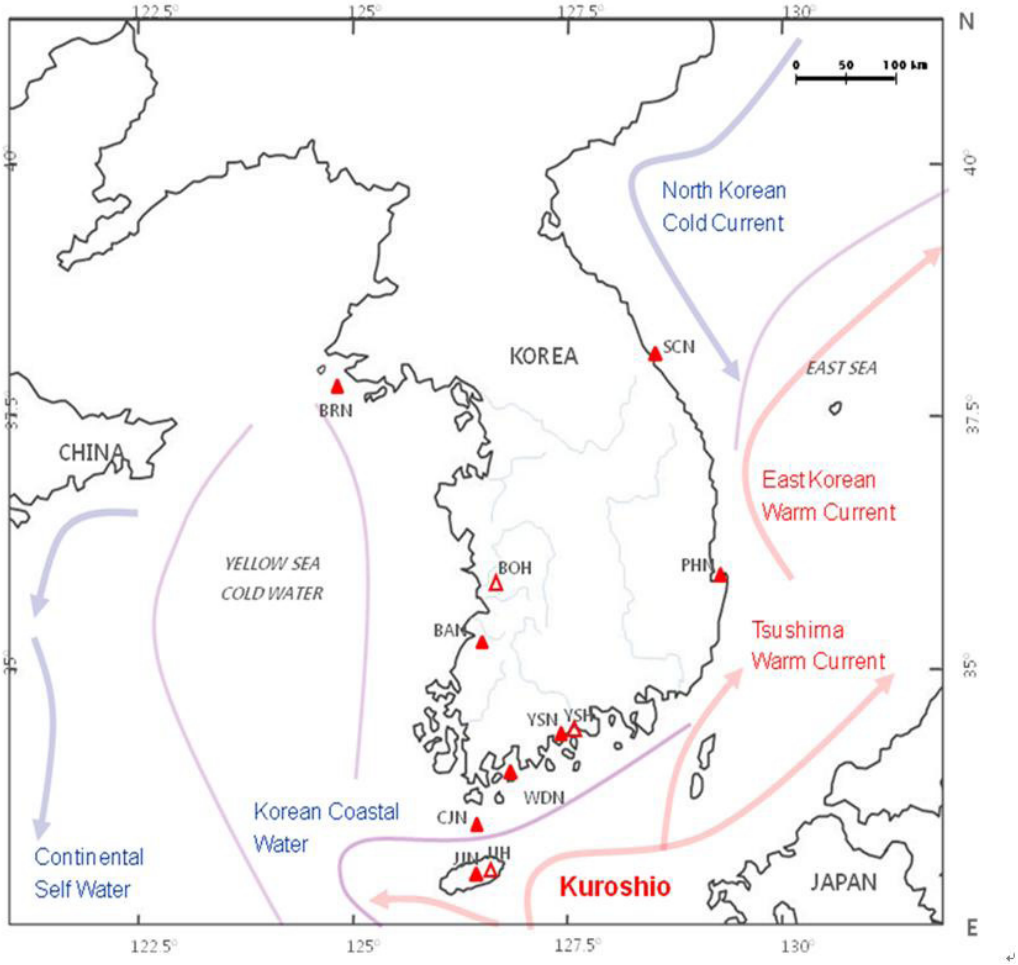

Figure 1. Approximate location of eight wild and three hatchery-reared sample sites of Pacific abalone (*Haliotis discus hannai*) in Korea and the main surface current system. Open triangles = hatchery-reared stock; closed triangles = wild population. YSH = hatchery cultured stock in Yeosu; BOH = hatchery cultured stock in Boryeong; JJH = hatchery cultured stock in Jeju; SCN = wild population in Sokcho; PHN = wild population in Pohang; BRN = wild population in Baekryeongdo; BAN = wild population in Buan; WDN = wild population in Wando; YSN = wild population in Yeosu; CJN = wild population in Chujado, and JJN = wild population in Jeju.

Table 1. Collection details for *Haliotis discus hannai* samples.

Sampling area (abbreviation)	Sample locality	Sample size	Collection date
Wild population			
Sokcho (SCN)	Eastern Sea; 38° 13' N, 128° 35' E	83	October 2003-September 2004
Pohang (PHN)	Eastern Sea; 36° 1' N, 129° 22' E	82	October 2003-September 2004
Baekryeongdo (BRN)	Western Sea; 37° 57' N, 124° 39' E	71	October 2003-September 2004
Buan (BAN)	Western Sea; 35° 24' N, 125° 53' E	87	October 2003-September 2004
Wando (WDN)	Southern Sea; 34° 13' N, 126° 36' E	96	October 2003-September 2004
Yeosu (YSN)	Southern Sea; 34° 45' N, 127° 40' E	90	October 2003-September 2004
Chujado (CJN)	Southern Sea; 33° 58' N, 126° 17' E	70	October 2003-September 2004
Jeju (JJN)	Southern Sea; 33° 14' N, 126° 32' E	37	October 2003-September 2004
Hatchery cultured strains			
Boryeong (BOH)	Western Sea; 36° 18' N, 126° 34' E	81	April 2004
Yeosu (YSH)	Southern Sea; 34° 55' N, 127° 35' E	92	April 2004
Jeju (JJH)	Southern Sea; 34° 48' N, 127° 5' E	53	April 2004

Mantle musculature samples were clipped and immediately preserved in 99.9% ethanol until DNA extraction was performed. Total genomic DNA for genotyping was extracted from each sample using the MagExtractor MFX-2100 (Toyobo, Osaka, Japan) automated DNA extraction system with a MagExtractor-Genomic DNA Purification kit (Toyobo). Extracted genomic DNA was stored at -20°C prior to the polymerase chain reaction (PCR) analyses.

Microsatellite genotyping

In total, 842 Korean Pacific abalones from eight wild and three hatchery-reared populations were genotyped using six Pacific abalone microsatellite loci. Hdh1321, Hdh513, Hdh512, and Hdh145, which were developed for *H. d. hannai* (Li et al., 2002), and Hdd114B and Hdd229, which were developed for *H. d. discus* (Sekino and Hara, 2001), were used to amplify the alleles by PCR. The same markers were used for comparisons with previous measurements from *H. d. hannai*. The 5'-end of the forward primer from each set of primers was labeled with fluorescent dye (6-FAM, HEX, or NED; Applied Biosystems, Foster City, CA, USA). The PCR amplification of the six microsatellite loci was performed using 10-µL solutions, containing 0.25 U Taq DNA polymerase, 10X ExTaq buffer, 2 mM dNTP mixture (Takara, Shiga, Japan), 2 µM each primer set and approximately 10 to 50 ng template DNA, using a PTC-0220 DNA Engine Dyad Peltier thermal cycler (MJ Research, Inc., Waltham, MA, USA). PCR conditions included an initial denaturation at 95°C for 11 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing for 1 min at the annealing temperature given in the literature (Sekino and Hara, 2001; Li et al., 2002), and extension at 72°C for 1 min, with a final extension at 72°C for 5 min.

For genotyping, 1-µL PCR product was added to 9-µL reaction mix containing formamide (Hi-Di Formamide, Applied Biosystems, Warrington, UK) and the GeneScan 400HD [ROX] size standard (ABI PRISM, Applied Biosystems). This mixture was denatured at 95°C for 2 min, and immediately chilled on ice. Fragment analyses of the reaction products were performed using an ABI 3130 Genetic Analyzer (Applied Biosystems) and the GeneMapper software (ver. 4.0; Applied Biosystems). To improve the accuracy of the allele size determinations, a control DNA sample was included with each set of samples for each run.

Data analysis

Statistical genetic analyses were conducted for eight wild and three hatchery-reared populations of *H. d. hannai*. The alleles were scored using GeneMapper version 4.0 (Applied Biosystems) with a size standard and an internal control for allele calling, and each allele was coded by its size in nucleotides (bp). A panel that included all of the alleles that were detected in the 842 individuals was created for each locus. The possible presence of null alleles and genotyping errors caused by stuttering and/or large-allele dropouts were tested using MICRO-CHECKER ver. 2.2.3 (1000 randomizations; van Oosterhout et al., 2004). Scoring and human error were estimated using duplicate analyses. Because this genetic analysis was based on only six microsatellites, the data were also tested to determine whether one or more microsatellites were under selection using the LOSITAN software, which is a selection-detection workbench that is based on a well-evaluated F_{ST}-outlier detection method (1000 randomizations; Antao et al., 2008). The standard genetic diversity statistics, including the number of alleles (N_A), allelic

and genotypic frequencies, size [S (bp)] and frequency (F) of the most common allele, number of observed unique alleles (U), observed heterozygosity (H_O), and expected heterozygosity (H_E), were determined for each local sample at each locus using the Genepop ver. 4.0 program (http://kimura.univmontp2.fr/~rousset/Genepop.htm) or ARLEQUIN version 3.1 (Excoffier et al., 2005). Because allele number is influenced by sample size, allele richness (A_R) was used for comparison, regardless of the sample size using the rarefaction method of FSTAT ver. 2.9.3.2 (http://www2.unil.ch/popgen/ softwares/fstat.htm). Differences in genetic diversity statistics between the cultured stocks and wild populations were tested using nonparametric analyses (Wilcoxon signed-rank test). The GENEPOP 4.0 software package was also used to assess the linkage disequilibrium between the pairwise loci and overall inbreeding coefficients (F_{IS}) (Weir and Cockerham, 1984) for each population and locus; F_{IS} can measure deviations from Hardy-Weinberg equilibrium (HWE) within a population. Deviations from HWE were tested using probability tests or exact tests according to the Markov-chain procedure of ARLEQUIN. The significance levels were adjusted for multiple tests using the sequential Bonferroni's correction (Rice, 1989). Because hatchery populations are often subjected to founder effects and bottlenecks that result in lower genetic diversity, the Bottleneck ver. 1.2.02 software program (Cornuet and Luikart, 1996) was used under the infinite allele model, stepwise-mutation model and two-phased mutation model with 1000 iterations to verify the existence of bottlenecks inferred by heterozygosity excess in the six samples. Significance was tested using the Wilcoxon signed-rank test. If significant results were obtained under all three models, it was concluded that the populations had experienced bottlenecks (Cornuet and Luikart, 1996).

A traditional test for population differentiation was performed by calculating the F_{ST} based on allelic identity (Weir and Cockerham, 1984) using ARLEQUIN, and significances were adjusted for multiple tests using sequential Bonferroni's correction (Rice, 1989). The strengths of the relationships between the geographical populations were estimated from the genetic distances based on the chord distance, D_{CE}, between all pairs of populations, which is one of the most efficient distance measures that can be used to determine accurate tree topology from allele frequency data (Cavalli-Sforza and Edwards, 1967). A phylogenetic tree was constructed for each replicated genetic distance matrix, with bootstrapping using the neighbor-joining (NJ) tree method of the POPULATION version 1.2.30 program (http://bioinformatics.org/~tryphon/populations/). Bootstrap values were calculated using 1000 replicates. The NJ tree was visualized using the Tree Explorer program (http://www.xmarks.com/site/evolgen.biol.metro-u.ac.jp/TE/TE_man.html). The genetic relationships between the samples were visualized by a principal component analysis (PCA) using GenAlEx 6.3 (http://www.anu.edu.au/BoZo/GenAlEx/), which was based on the covariance matrix of gene frequencies, and MDS (multidimensional scaling) using SPSS 13.0, which was based on pairwise F_{ST} values. A hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992) was used to partition genetic variance between the subsamples within groups (F_{SC}) and among groups (F_{CT}) using ARLEQUIN ver. 3.1 (Excoffier et al., 2005), and the significances of AMOVA components were tested using 1000 permutations. The correlation between genetic distance and geographical distance was evaluated using the Mantel test with GenAlEx 6.3. The patterns of the wild population structure were further investigated using the model-based Bayesian clustering procedure in STRUCTURE version 2.2 (Pritchard et al., 2000), which assigns individuals to K populations based on their multilocus genotypes. STRUCTURE was run for K = 1-8 using a burn-in length of 50,000 and a run of 50,000 steps. All runs were repeated in triplicate at each K, and the results were consistent across runs.

RESULTS

Genetic variability

The genotypes for the six microsatellite loci were determined for the 842 Pacific abalones from Korea. The six primer sets were polymorphic across all of the Korean Pacific abalone populations that were studied, and the levels of polymorphism (numbers and frequencies of alleles) varied depending on the locus. Reruns were conducted for approximately 20% of all individuals to ensure the reproducibility of allele scoring, which produced identical results for each trial. Samples that failed to amplify after the rerun were not included, thereby making it unlikely that poor DNA quality affected the results.

We found no indication of allele scoring errors as a result of stuttering or large allele dropout, but the possibility that all loci may have been influenced by one or more null alleles was acknowledged in the populations studied after checking their microsatellite genotypes with the MICRO-CHECKER program. However, all six loci were used in this study because no null alleles affected all populations. A Lositan analysis did not detect any outlier loci with excessively high or low F_{ST} values compared to the neutral expectations, indicating that all of the microsatellites used were not likely to be subject to selection.

The measures of genetic diversity for each population as calculated from the observed allele distribution are presented in Table 2. A total of 308 alleles were detected across the six loci. The mean N_A and mean A_R ranged from 18.8 to 31.3 and 16.8 to 24.6, respectively, with an average of 51.3 alleles per locus. No population had a diagnostic allele. The mean H_E and H_O per locus ranged from 0.858 to 0.925 and 0.667 to 0.828, respectively. Differences in genetic diversity were reflected in the wild and hatchery samples. For the comparison between hatchery stocks and wild populations, A_R was used because N_A varies widely depending on the sample size of populations. The averages of A_R values in the three hatchery stocks (16.8-21.7) were relatively lower than those in the eight wild populations (21.4-24.6), but the differences were not significant (Wilcoxon signed-rank test, $P > 0.05$). A comparison of mean H_E values revealed that the average H_E value of the wild populations tended to be slightly higher than that of the hatchery samples (0.917 *vs* 0.899, respectively; Wilcoxon signed-rank test, $P > 0.05$). The inbreeding coefficient (F_{IS}) values of the cultured stocks did not significantly differ from those of the wild populations (Wilcoxon signed-rank test, $P > 0.05$) (Table 2). In contrast, there was a tendency towards a higher frequency of private alleles within the wild stocks; 53 of 80 unique alleles were found to be unique among the wild populations (Table 2), which were all observed at low frequencies. Because the numbers of individuals that were analyzed differed between the populations, the results should be interpreted with caution. Despite these differences in genetic diversity, no clear difference in the average measurements was observed between the wild and hatchery samples.

Each locus had one relatively common allele that was shared across populations despite the population differences in the allele frequencies. An analysis of the most frequent allele showed that variable numbers of alleles were observed at the six loci between the eight wild and three hatchery populations (Table 2). No similar common alleles were observed across the wild and hatchery population sets at all loci. The sample size could be an altering factor in the number of unique alleles or low frequency alleles, but it should not affect the frequencies of the most common alleles. Thus, the different common alleles among the populations were strongly suggestive of genetic differentiation. An examination of the pair-wise linkage disequilibrium revealed that all six microsatellite loci were in linkage equilibrium.

Table 2. Allelic variability observed at six microsatellite loci in eight wild and three hatchery populations of *Haliotis discus hannai*.

Locus	Statistics	Population (N)										
		Wild								Hatchery		
		SCN (83)	PHN (82)	BRN (71)	BAN (87)	WDN (96)	YSN (90)	CJN (70)	JJN (37)	YSH (92)	BOH (81)	JJH (53)
Hdh1321	N_A (U)	28(1)	36(1)	38(3)	41(2)	37(1)	41(0)	41(3)	30(0)	34(2)	38(1)	27(5)
	A_R	23.6	26.7	27.9	29.8	26.7	27.4	31.6	30.0	24.6	28.0	22.7
	S (bp)	292	296	326	312	300	300	300	320	300	326	300
	F	0.151	0.079	0.141	0.092	0.130	0.139	0.121	0.095	0.212	0.148	0.330
	H_E	0.920	0.958	0.952	0.948	0.942	0.955	0.946	0.898	0.941	0.944	0.837
	H_O	0.855	0.705	0.901	0.954	0.604	0.818	0.643	0.614	0.785	0.790	0.674
	F_{IS}	0.071	0.201*	0.054	-0.007	0.260*	0.148	0.328*	0.232*	0.223*	0.194*	0.135
Hdd114B	N_A (U)	27(2)	33(2)	30(0)	28(2)	31(1)	32(1)	28(0)	23(0)	25(1)	26(2)	18(0)
	A_R	20.0	27.4	25.3	23.6	24.4	26.2	23.9	23.0	21.5	22.4	15.7
	S (bp)	218	218	214, 218	226	218	218	226, 228	218	218	220	154
	F	0.145	0.098	0.077	0.109	0.130	0.100	0.107	0.284	0.125	0.117	0.302
	H_E	0.939	0.942	0.941	0.958	0.949	0.945	0.958	0.964	0.923	0.940	0.867
	H_O	0.855	0.829	0.901	0.908	0.854	0.844	0.814	0.946	0.891	0.889	0.868
	F_{IS}	0.090	0.120	0.042	0.022	0.100	0.107	0.046	0.091	0.035	0.055	-0.002
Hdd229F	N_A (U)	26(2)	28(0)	23(1)	24(0)	28(0)	23(0)	27(0)	18(0)	23(0)	24(0)	20(2)
	A_R	22.2	23.2	20.6	20.3	22.0	18.3	22.3	18.0	19.5	20.7	18.9
	S (bp)	202	202	190, 192	190, 200	188	188	172	192	172	198, 200	196
	F	0.133	0.110	0.120	0.115	0.161	0.150	0.129	0.176	0.130	0.130	0.170
	H_E	0.941	0.948	0.938	0.937	0.935	0.917	0.938	0.901	0.934	0.931	0.923
	H_O	0.602	0.800	0.804	0.839	0.663	0.678	0.800	0.892	0.574	0.854	0.615
	F_{IS}	0.261*	0.174	0.150	0.105	0.200*	0.256*	0.148	0.080	0.230*	0.130	0.253*
Hdh513	N_A (U)	47(2)	59(7)	42(2)	40(0)	53(5)	54(3)	49(2)	33(0)	46(5)	42(1)	26(2)
	A_R	35.3	42.0	31.9	30.1	35.7	36.6	37.9	33.0	31.8	32.3	23.7
	S (bp)	378	384	374, 378	378	372, 400	374	372, 374, 412	388	370	374	198, 374, 388
	F	0.054	0.055	0.070	0.080	0.063	0.100	0.057	0.081	0.114	0.068	0.113
	H_E	0.971	0.981	0.966	0.966	0.972	0.970	0.977	0.971	0.960	0.969	0.942
	H_O	0.828	0.878	0.831	0.751	0.602	0.867	0.843	0.973	0.880	0.826	0.662
	F_{IS}	0.045	0.136	0.141	0.192*	0.276*	0.108	0.135	-0.002	0.083	0.145	0.180*

Continued on next page

Table 2. Continued.

Locus	Statistics	Population (N)										
		Wild								Hatchery		
		SCN (83)	PHN (82)	BRN (71)	BAN (87)	WDN (96)	YSN (90)	CJN (70)	JJN (37)	YSH (92)	BOH (81)	JJH (53)
Hdh145F	N_A (U)	9(2)	7(0)	8(1)	7(0)	7(0)	8(1)	8(2)	7(0)	8(1)	7(0)	5(0)
	A_R	7.8	6.8	7.4	7.0	7.0	7.4	7.0	7.0	7.3	6.8	5.0
	S (bp)	134	134	134	136	134	136	136	136	136	134	134
	F	0.301	0.323	0.394	0.241	0.250	0.328	0.321	0.486	0.266	0.247	0.396
	H_E	0.800	0.780	0.749	0.813	0.820	0.799	0.767	0.717	0.807	0.823	0.726
	H_O	0.759	0.671	0.690	0.644	0.808	0.550	0.700	0.441	0.587	0.741	0.679
	F_{IS}	0.052	0.140	0.080	0.209*	0.137	0.215*	0.109	0.219*	0.214*	0.100	0.155
Hdh512	N_A (U)	25(0)	25(0)	24(1)	22(0)	25(1)	27(0)	25(2)	18(0)	24(2)	22(0)	17(3)
	A_R	19.5	21.5	20.2	18.7	20.8	21.4	21.2	18.0	19.4	19.8	14.7
	S (bp)	112	112	112	104	104	108	112	104	118	112	116
	F	0.217	0.159	0.183	0.190	0.146	0.133	0.164	0.162	0.168	0.136	0.255
	H_E	0.911	0.931	0.919	0.918	0.932	0.935	0.931	0.923	0.925	0.939	0.854
	H_O	0.791	0.695	0.618	0.870	0.829	0.796	0.800	0.838	0.850	0.528	0.504
	F_{IS}	0.154	0.255*	0.229*	0.110	0.131	0.163	0.150	0.174	0.101	0.285*	0.295*
Mean	N_A (U)	27.0(1.5)	31.3(1.7)	27.5(1.3)	27.0(0.7)	30.2(1.3)	30.8(0.8)	29.7(1.5)	21.5(0.0)	26.7(1.8)	26.5(0.7)	18.8(2.0)
	A_R	21.4	24.6	22.2	21.6	22.8	22.9	24.0	21.5	20.7	21.7	16.8
	H_E	0.914	0.923	0.911	0.923	0.925	0.920	0.920	0.896	0.915	0.924	0.858
	H_O	0.782	0.763	0.791	0.828	0.727	0.759	0.767	0.784	0.761	0.771	0.667
	F_{IS}	0.112	0.171	0.116	0.105	0.184	0.166	0.153	0.132	0.148	0.151	0.169

Number of samples (N), number of alleles per locus (N_A), allelic richness (A_R), size of the most common allele [S (bp)], frequency (F) of the most common allele, number of unique alleles (U), expected heterozygosity (H_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}) are given for each population and locus. *Not in conformity with Hardy-Weinberg equilibrium (P < 0.008, Bonferroni-corrected value).

Of the 66 independent loci that were examined by the Hardy-Weinberg tests, 22 (33.3%) exhibited significant deviations from HWE after adjusting the P values across the six loci using the sequential Bonferroni method for multiple observations ($P < 0.008$) (Wilcoxon, 1945). However, these significant deviations were not distributed evenly among the samples or loci. No significant deviation was detected at the Hdd114B locus. Deviation from equilibrium was observed at five loci in the wild and hatchery samples in 14 and 8 cases, respectively. No locus or sampling site had these departures as a result of a deficit in heterozygosity, and no excess heterozygosity was detected.

The bottleneck analysis showed no evidence of a recent bottleneck for any of the eight wild and three hatchery samples ($P > 0.05$), indicating that none of these samples had recently experienced bottlenecks.

Genetic differentiation among populations

Small but highly significant genetic differentiation was observed among the populations ($F_{ST} = 0.017$, $P < 0.01$). Table 3 presents the pattern of differentiation between the 11 abalone populations, which was observed by comparing D_{CE} and mean pairwise F_{ST} values based on the microsatellite data. Combinations of the 11 populations consistently yielded highly significant F_{ST} values (ranging from 0.001 to 0.084) for most cases ($P < 0.01$); however, five combinations between the wild populations were not significant ($P > 0.05$) in case where the geographical distances were relatively small. Surprisingly, three combinations between the wild and hatchery populations (BOH-BAN, BOH-WDN, and BOH-YSN) were also not significant ($P > 0.05$) (Table 3). Significant genetic differentiation was observed among both the wild populations ($F_{ST} = 0.012$, $P < 0.01$) and hatchery stock samples ($F_{ST} = 0.030$, $P < 0.01$), but, no significant differentiation was found between the wild and hatchery populations. The hierarchical analyses (AMOVA) showed that most of the genetic differentiation was distributed among populations within groups, whereas differentiation between the two groups was low ($F_{CT} = 0.002$, $P > 0.05$; Table 4). The D_{CE} genetic values between the hatchery stocks (ranging from 0.012 to 0.027) tended to be larger than those between the wild populations (ranging from 0.006 to 0.025), and the genetic distance values between the wild and hatchery populations varied from 0.007 to 0.037. The largest differentiation among the wild populations was between JJN and BRN ($F_{ST} = 0.042$, $P < 0.05$), whereas the smallest divergence occurred between PHN and SCN ($F_{ST} = 0.001$, $P > 0.05$).

Table 3. D_{CE} distance (below the diagonal) and mean F_{ST} estimates (above the diagonal) between each pair of eight wild populations and three hatchery stocks of *Haliotis discus hannai*.

Population	SCN	PHN	BRN	BAN	WDN	YSN	CJN	JJN	YSH	BOH	JJH
SCN	-	0.0010	0.0196*	0.0182*	0.0164*	0.0184*	0.0204*	0.0342*	0.0200*	0.0179*	0.0545*
PHN	0.0067	-	0.0155*	0.0116*	0.0095*	0.0122*	0.0160*	0.0271*	0.0158*	0.0126*	0.0496*
BRN	0.0140	0.0125	-	0.0117*	0.0109*	0.0176*	0.0270*	0.0415*	0.0201*	0.0071*	0.0432*
BAN	0.0141	0.0111	0.0074	-	0.0015	0.0038	0.0071*	0.0190*	0.0084*	0.0026	0.0522*
WDN	0.0137	0.0110	0.0092	0.0065	-	0.0025	0.0064*	0.0205*	0.0062*	0.0033	0.0447*
YSN	0.0136	0.0106	0.0095	0.0062	0.0061	-	0.0027	0.0131*	0.0050*	0.0039	0.0521*
CJN	0.0160	0.0134	0.0122	0.0092	0.0094	0.0085	-	0.0139*	0.0095*	0.0117*	0.0591*
JJN	0.0249	0.0194	0.0184	0.0130	0.0131	0.0123	0.0145	-	0.0225*	0.0222*	0.0843*
YSH	0.0159	0.0148	0.0119	0.0111	0.0101	0.0100	0.0113	0.0192	-	0.0086*	0.0477*
BOH	0.0158	0.0124	0.0082	0.0067	0.0068	0.0072	0.0114	0.0142	0.0116	-	0.0454*
JJH	0.0298	0.0275	0.0244	0.0281	0.0264	0.0272	0.0273	0.0366	0.0263	0.0271	-

D_{CE} distance (Cavalli-Sforza and Edwards, 1967) and pairwise F_{ST} (Weir and Cockerham, 1984) are measures of genetic distance and genetic differentiation between populations, respectively. *$P < 0.009$ from a multi-locus test with no genetic differentiation in population pair after corrections for multiple comparisons (Bonferroni's correction). For population abbreviations, see Table 1.

Table 4. Analysis of the proportion of genetic variation distributed between the two groups of wild and hatchery populations and distributed among populations within these groups.

Statistics	Among populations within groups (wild and hatchery populations)	Between groups (wild and hatchery populations)	Total differentiation
F	$F_{SC} = 0.017$**	$F_{CT} = 0.002^{NS}$	$F_{ST} = 0.017$***

Hierarchical analyses of genetic differentiation among populations were applied using Weir and Cockerham's (1984) estimators of *F* statistics. Table-wide significance levels were applied in the k = 3 tests, using the sequential Bonferroni technique (Rice, 1989). **P < 0.01; ***P < 0.001; NS = not significant (P ≥ 0.05).

The NJ tree was constructed on the basis of the D_{CE} distances (Figure 2). This tree showed that two wild populations that originated from the eastern coast of Korea (SCN and PHN) formed one cluster, and the other six wild populations from the western and southern coasts of Korea (BRN, BAN, WDN, YSN, CJN, and JJN) formed the other one. Two hatchery samples (YSH and BOH) were grouped with the latter cluster, and one hatchery sample (JJH) was separated from the clusters of wild population samples by a long branch-length, indicating a high level of genetic divergence. These findings were also obvious in the PCA scatter plots (Figure 3) and clearly revealed three major sets. The six wild (BRN, BAN, WDN, YSN, CJN, and JJN) samples and two hatchery samples (YSH and BOH) were assigned to a bigger single set, and the other two wild samples (SCN and PHN) and one hatchery sample (JJH) were included in two additional separate sets. The genetic relationships that were suggested by the pairwise F_{ST} values were clearly visualized in the multidimensional scaling analysis (Figure 4). The MDS results (stress = 0.056; RSQ = 0.991) revealed that the two hatchery stocks, with the exception of JJH, markedly differed from the others and were similar to the wild populations; additionally, two wild samples (SCN and PHN) differed from the other wild samples. This genetic structuring was supported by AMOVA (P < 0.05). Further clustering analyses among the wild populations were performed using the Bayesian clustering method with the STRUCTURE software under the condition of increasing numbers of inferred clusters (K = 1 to 8), and the results suggested the most likely population structure involved two clusters (Figure 5). Two wild samples (SCN and PHN) showed markedly different plots compared to the other wild samples. This pattern of structuring was similar to those of the phylogenetic tree, PCA and MDS analyses. In summary, these results suggest that genetic divergence between the two geographical populations (i.e., BRN-BAN-WDN-YSN-CJN-JJN and SCN-PHN) may have taken place.

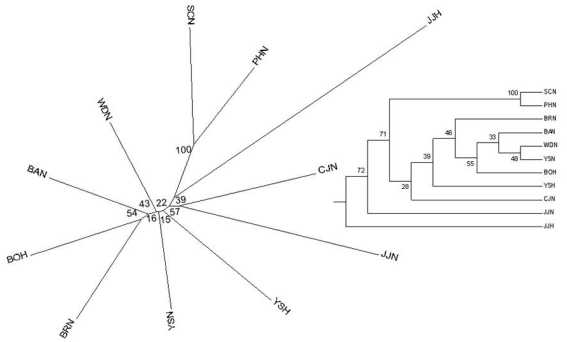

Figure 2. Neighbor-joining tree and phenogram (inset) derived from D_{CE} distance matrix for eight wild and three hatchery populations of *Haliotis discus hannai*. The numbers refer to percentage bootstrap values generated from 1000 replications of resampled loci. For population abbreviations, see Table 1.

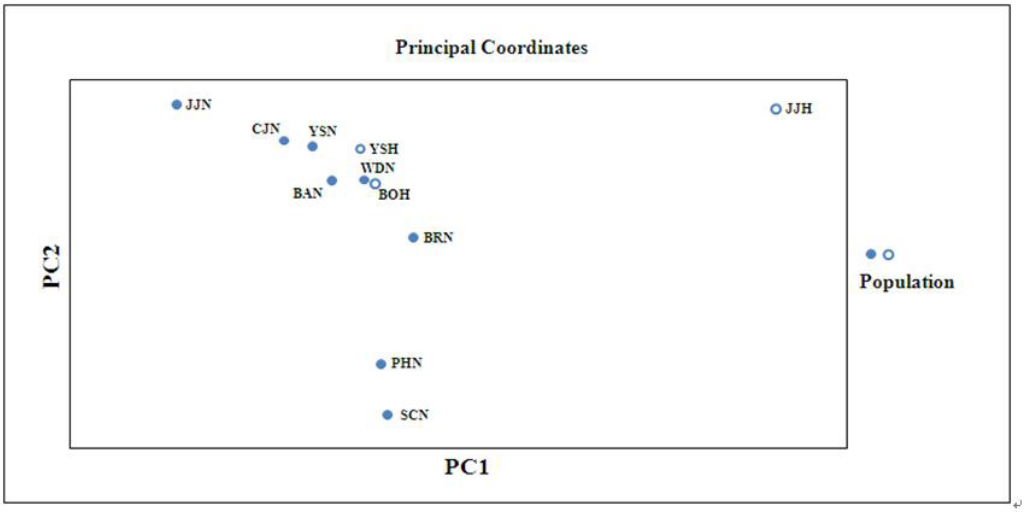

Figure 3. Principal component (PC1 and PC2) analysis plotting the relationships of the *Haliotis discus hannai* wild and hatchery population samples studied, based on D_{CE} genetic distances (Cavalli-Sforza and Edwards, 1967). Closed circle = wild population; open circle = hatchery population; for population abbreviations, see Table 1.

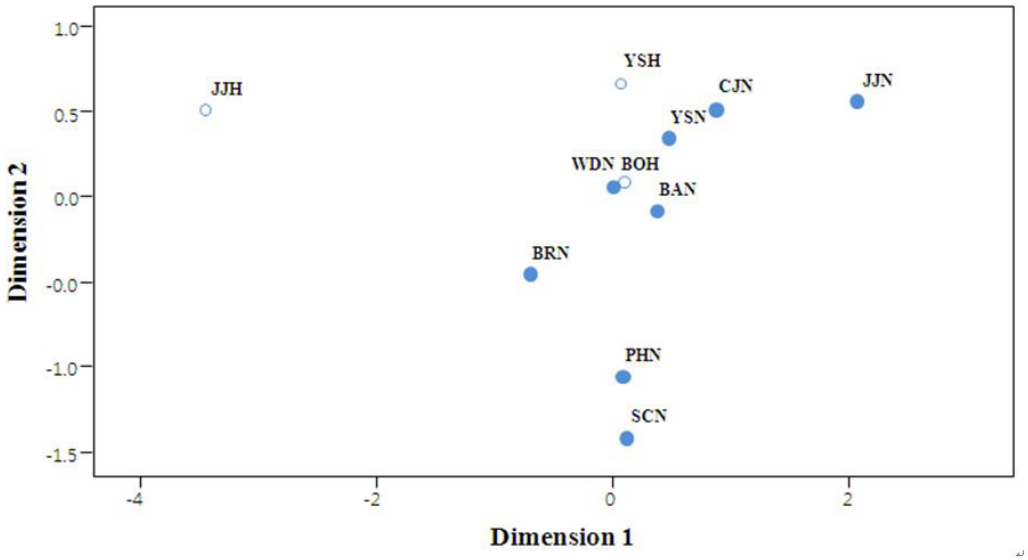

Figure 4. Multi-dimentional scaling plot of four wild populations and two cultured stocks of the Korean Pacific abalone for genetic distribution based on pairwise F_{ST} values. Closed circles = wild population; open circles = hatchery population. For population abbreviations, see Table 1.

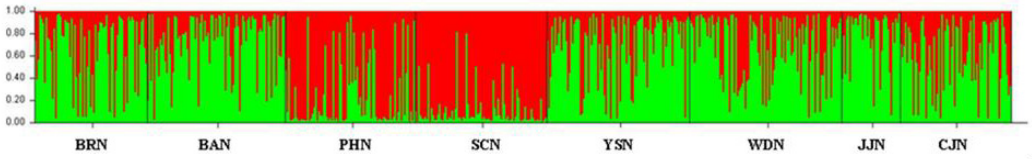

Figure 5. Estimated genetic subsamples of eight wild populations of *Haliotis discus hannai*. The graph is based on the proportion of individuals per population in the inferred clusters according to STRUCTURE 2.2. Each of the two colors represents a different genetic cluster, and black lines separate the populations. Populations are labeled below the figure. The vertical line indicates the probability that each individual belongs to the inferred cluster. For population abbreviations, see Table 1.

Overall, the genetic differences were not explained by geographical distance because the isolation-by-distance model of genetic structure showed no significant correlations ($P > 0.05$).

DISCUSSION

Although the abalone fishery in Korea is believed to be depleted, high levels of genetic variability were detected in all populations (average $N_A = 27$; average $H_E = 0.912$) because the allelic diversities and heterozygosities were considerably high in both the wild and hatchery populations, and little difference was found between them (Table 2). Molecular genetic diversity has been associated with life history traits that reflect habitat types (DeWoody and Avise, 2000). High levels of genetic diversity seem to be common in marine bivalves because of the large population sizes and high nucleotide mutation rates (Zhan et al., 2007). With an equal number of microsatellite markers, a similar high genetic variability in wild and hatchery populations was also reported for the Japanese and Korean Pacific abalone, respectively (Li et al., 2004; An et al., 2011a). Genetic variability is an important attribute of domesticated species, because those with higher levels of variation are the most likely to have high genetic variances for productive traits (Hurt and Phillip, 2004). Several studies have shown that aquaculture practices reduce genetic variability in hatchery-reared stocks of various fish species (Meier et al., 2011). The present study, however, revealed little difference between the wild and hatchery populations with regard to allelic variation, heterozygosity or inbreeding coefficients, indicating that high levels of genetic variation have been preserved in the hatchery populations. In general, the decline of genetic variation in the hatchery stocks may be caused by the increased effect of genetic drift resulting from the use of a small number of parental individuals (Norris et al., 1999). In this study, however, these founder effects may have been insignificant because there was no evidence of a bottleneck in the hatchery stocks. Besides founder effects, the genetic changes that were observed in the hatchery stocks compared to the wild populations were likely due to the artificial and natural selection that exists in the aquaculture environment (Mjolnerod et al., 1997). However, this selection could not be reflected using neutral markers such as the microsatellites in our study. The only indication of possible stock heterogeneity in our data is the tendency towards a lower proportion of private or unique alleles among the hatchery-reared Pacific abalone, which may only reflect the fact that the hatchery-reared stock is genetically isolated, whereas the wild one may be infiltrated by individuals from neighboring populations.

Significant deviations from HWE were observed at all loci with the exception of

Hdd114B and across all samples. This phenomenon, due to a high positive F_{IS} value, is relatively common for marine organisms (Hara and Sekno, 2007; Han et al., 2012). In our case, the heterozygote deficiency may have been due to one or several causes. The Wahlund effect, which is the result of translocations or stocking practices and inbreeding by mating between relatives, may have contributed to the deviations from HWE that were observed in this study. In addition, the null alleles that were observed with the microsatellite loci may be a more plausible reason for the deviation. In our study, null alleles were observed for five of the six loci; no significant deviation from HWE was observed at the Hdd114B locus, which does not have a null allele.

Although the results regarding the genetic variability between the wild and hatchery populations provided important information for the aquaculture of this species, the high levels of genetic diversity that were observed in the hatchery stocks must be regarded cautiously. Based on the findings of high levels of genetic diversity in both the wild and hatchery populations, it seems that the present wild population of Pacific abalone in Korea still maintains normal levels of genetic diversity, and its genetic variability is relatively unaffected despite its decline in numbers during the past decades due to overfishing and habitat destruction. However, there is no genetic diversity information on the wild Pacific abalone prior to the population decline. Thus, monitoring of the genetic diversity of historical and contemporary samples should be performed to gain a more comprehensive understanding of the genetic variation of this species. Our findings of higher genetic diversity in the hatchery-reared population contrasted with the usual perception that domestic populations should harbor lower genetic diversity (Karaiskou et al., 2009). Other studies, however, found no differences between the genetic diversities of hatchery-reared and wild populations of Pacific oyster (Yu and Li, 2007). Furthermore, self-seeding may be related to the high genetic diversity of overexploited abalone populations (Miller et al., 2009). Thus, like many marine invertebrates, some level of inbreeding and heterozygote deficits were observed in the wild abalone populations. Given that the source of higher proportions is likely to vary in time and space in depleted populations, the end result may be a local population with a more diverse genetic composition than those populations that are largely self-recruiting. Although over-fishing in itself will not result in increased genetic diversity, the changed ratio of migrant to local larvae may well result in an apparently higher genetic diversity in the recovering population (Miller et al., 2009).

The overall genetic differentiation among these 11 Pacific abalone populations was weak but significant. Our results showed the existence of genetic heterogeneity in the three major portions (i.e., BRN-BAN-BOH-WDN-YSH-YSN-CJN-JJN, SCN-PHN and JJH), which was based on the F_{ST} value, phylogenetic tree clustering, PCA and MDS analyses, structure, and AMOVA. The patterns of microsatellite divergence in the BRN, BAN, WDN, YSN, CJN and JJN populations indicated that Pacific abalone populations around the western and southern coasts of Korea are genetically grouped. These results suggest that sea currents are responsible for the high gene flow. A portion of the Tsushima Warm Current (TWC) that branches off the Kuroshio around the southern coast runs into the Yellow Sea (Figure 1). This current may transport larvae to the eastern part of the Yellow Sea. Thus, the BRN and BAN populations of the western coast of Korea are more similar to the southern coast populations. A significant amount of genetic differentiation was also detected between the SCN and PHN populations (East Sea populations) and the pooled western and southern wild populations, suggesting low or restricted dispersal and gene flow between these regional populations. Two

wild population samples (SCN and PHN) are located at the subpolar front, which is where the cold and warm currents of the East Sea meet (Figure 1). The subpolar front, which extends along the coast of Japan before turning sharply at the Noto Peninsula frontal region towards the center of the East Sea, has a close relationship with the TWC and cold-water currents, including the Northern Korea Cold Current and the Liman Current (Ichikawa and Beardsley, 2002). Therefore, the genetic differentiation of SCN and PHN from the other wild populations may be explained by the balanced effects of the cold (Northern Korea Cold Current and Liman Current) and warm (TWC) water currents in the East Sea. The significant population structuring that was observed in the East Sea areas of Korea was also found in other marine species, such as olive flounder (*Paralichthys olivaceus*) (Kim et al., 2010b) and Pacific cod (*Gadus macrocephalus*) (Kim et al., 2010a). However, in this study, the genetic differences were not explained by geographical distance and may be caused by factors such as the hydrographic complexity of the habitats and biological factors, including historical dispersal dynamics.

The hatchery JJH population showed marked reductions in the mean number of alleles, allelic richness and heterozygosity when compared with the wild populations and the other hatchery populations. Therefore, the pair-wise F_{ST} value, phylogenetic tree clustering and PCA and MDS analyses showed that the JJH population is genetically distinct from the other wild and hatchery populations. This finding is in agreement with the fact that the hatchery stocks are characterized by having reduced genetic variation due to the random genetic drift that exists in the aquaculture environment. Alternatively, the possibility that *H. d. discus* (Camacjeonbok), which is a sister subspecies of *H. d. hannai* (Cham-jeonbok), constitutes this population cannot be excluded because *H. d. discus* is a highly valuable fishery resource in Jeju, Korea, and clear discrimination based on the morphological characteristics between the two species is difficult (An et al., 2005; Hara and Sekino, 2005). Previously, it was reported that microsatellite markers developed from *H. d. hannai* provided high resolution for the analysis of the genetic differences between *H. d. hannai* and *H. d. discus* populations (Hara and Sekino, 2005).

In addition, no genetic differentiation between the two groups of wild and hatchery populations was detected in the present study. This result is incongruent with a previous study that reported significant differentiation between one wild population and one hatchery population of Korean Pacific abalone (An et al., 2010a,b). Considering the fact that the previous study was limited by the number of populations that were screened, these results suggest that there may be merit in further investigations of the genetic structure using a larger number of sample collections. In the present study, the increase in sampling coverage allowed for a better-resolved genetic structure and enabled us to conclude that the genetic subdivision along the Korean coast is weak and that there is no differentiation between the two groups of wild and hatchery populations. Unlike the present study, previous studies involving Pacific abalone from China and Japan revealed the presence of genetic divergence between the wild and hatchery populations (Sekino et al., 2005; Hara and Sekino, 2007; Li et al., 2004, 2007). Given the intensity of Pacific abalone aquaculture activities and stock enhancement programs in Korea, the possibility that they may have partially contributed to the population samples cannot be excluded. The Pacific abalone populations in Korea have been exposed to heavy commercial fishing pressure in the past. Thus, intensive reseeding and stocking efforts have been conducted since the late 1990s. In recent years, government-sponsored hatcheries of Pacific abalone have released a number of hatchery-reared juveniles into the sea. This could explain

the apparent genetic similarities between the wild and hatchery populations that were observed in this study. Individuals of hatchery origin commonly co-occur with wild individuals, which pose increased risks of introgression. Recently, it was demonstrated that stocking domesticated marine organisms in wild populations greatly affected their genetic integrity by modifying the nature of genetic diversity within populations and by reducing the level of population genetic differentiation (Marie et al., 2010). Although the relative fitness of hatchery and wild Pacific abalone has not been directly assessed, it seems clear that gene flow from hatchery to wild populations is large enough to impact the genetic constitution of the wild population. From the point of view of biodiversity, the close genetic relationships between the wild and hatchery populations may be problematic because a large number of genetically distinct populations are influenced by Pacific abalones from one single gene pool due to stocking. Although we have no information regarding the amount of genetic change that may have occurred since the enhancement program was initiated, we note that the present regime is expected to be generally less successful from a conservative perspective. Therefore, these programs should be carried out with caution.

In conclusion, no detailed information is available to date on the genetic structure and diversity between and within the cultured stocks and wild populations of Pacific abalone; however, this information is critical for the establishment of suitable guidelines for resource management and selective breeding. This preliminary study using microsatellite loci showed no genetic differentiation between the two groups of hatchery and wild populations. This result will be useful for ongoing breeding and stock enhancement programs. We also observed the existence of genetically structured populations resulting from the cooperative effects of multiple factors. The genetic differences between geographical populations may provide a wide range of options for selective breeding and resource management for the sustainable use of this species. Quite clearly, future management plans must take into account the preservation of the genetic diversity and structure of populations of wild Pacific abalone. Efforts should be made to avoid the genetic mixing and subsequent breakdown of stock differentiation, which impair local adaptation in wild populations. Further studies must be conducted to provide more precise information on the dynamics of introgression by stocking.

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