Original Article

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Genetic Differences Between Hatchery Stocks and Natural Populations in Pacific Abalone (*Haliotis discus*) Estimated Using Microsatellite DNA Markers

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Abstract

Genetic variations within and between nine hatchery stocks and seven natural populations of abalone including Ezo-abalone (*Haliotis discus hannai*) and Kuro-abalone (*H. d. discus*) were assayed with nine microsatellite markers. Marked reductions of genetic variability in the hatchery stocks were recognized in the allelic diversity and mean heterozygosity compared with the natural populations. Thirteen of 16 significant HWE deviations in hatchery stocks revealed heterozygotes excess, while all natural populations did not show such a tendency. Highly significant F_{ST} values were observed for all cases between the hatchery stocks, and between the hatchery stocks and natural populations. Genetic distance (D_A) between each hatchery stock and the geographically proximal population (mean ± SD, 0.108 ± 0.035) were similar to those estimated for between the natural Ezo-abalone and Kuro-abalone (0.101 ± 0.021). The self-assignment test, which allocated individuals to their own stock with a high success rate, provided evidence of solid genetic differences among the nine hatchery stocks. These results suggests that the allelic composition and diversity in the natural populations was not necessarily reflected in the hatchery stocks owing to population bottleneck and genetic drift through seedling process, and thus the seedling and stocking practice of these hatchery stocks should take much notice of the results to conserve the genetic diversity of natural populations.

Keywords: Genetic diversity — genetic relationship — *Haliotis discus* — hatchery stock — microsatellite markers — Pacific abalone

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Introduction

Production of many important fishery species has been decreasing as a result of destruction of habitats suitable for spawning and nursing environment, and over-fishing. To compensate for the reduced fishery resources, enhancement practices have been intensively continued to release hatchery stock into natural coastal areas. A reduced genetic diversity was observed in most hatchery stocks and has been related to a loss of adaptations for new environments (Allendorf and Ryman, 1987), and thus genetic monitoring for hatchery stocks and natural populations is recommended to preserve genetic variations in natural populations (FAO, 1993).

The Pacific abalone *Haliotis discus* species, including a cold-current type called Ezo-abalone and a warm-current type called Kuro-abalone, is widely distributed in coastal areas of East Asia (Ino, 1952) and is the most important abalone species for commercial fisheries resources owing to its high market value. Recent annual landing of this species has been reduced to less than the half volume (2004-2200 tons, Ministry of Agriculture, Forestry, and Fisheries, Japan, 2005) compared to the maximum catch level (1970-6500 tons). This has led to an increase in hatchery abalone production from the 1980s, and a large amount of hatchery abalone has been stocked intensively in coastal areas across Japan every year. In this situation, the genetic impact of stocked hatchery abalone on the natural resources is a growing concern for a sustainable fishery. Genetic variability of hatchery stocks in Pacific abalone has been researched using allozyme and microsatellite markers. Allozyme analysis allowed differentiation of allelic diversity between hatchery stocks and natural populations, but failed to estimate genetic compositions of hatchery stocks owing to the limited number of available poly-

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morphic loci (Kijima et al., 1992). Six microsatellite markers could detect sensitively the reductions of genetic variability on allelic diversity and mean heterozygosity (Li et al., 2004), leaving the possibility that the use of additional loci would provide a more precise estimation of genetic relatedness between hatchery stocks. This study was conducted to assay the genetic diversity within and between hatchery stocks and natural populations of Pacific abalone both at population and individual levels based on nine microsatellite DNA markers.

Materials and Methods

Abalone Population Samples. Pacific abalone (Ezo- and Kuro-abalone) samples surveyed in this study were collected from the geographical sites shown in Figure 1. Sampling locality with an abbreviated population name, date of sampling, and sample size of each population and founding records are shown in Table 1. The natural Ezo-abalone populations were derived from Iwanai in Hokkaido (NEA), Omoe (NEB), and Hirota (NEC) in Iwate Prefecture ,and Kinkasan Island in Miyagi Pref. (NED). The natural Kuro-abalone populations were collected from Ijika in Mie Pref. (NKE), Mikuni in Fukui Pref. (NKF) and Ryotsu in Niigata Pref. (NKG). All hatchery stocks (nine stocks) screened in this study were created using natural captives sampled from coastal areas geographically proximal to the sampling sites of the natural populations (Figure 1). The HEA and HEE stocks were founded using parents maintained in the hatchery over several generations, and the HEC was both natural caught parents and brood-stock reproduced in the hatchery. The other stocks (HEB, HED, HEF, HKG, HKH, and HKI) were composed of first-generation offspring. Unfortunately, there are no detailed records for all the stocks regarding how many number of parents actually contributed to the production of offspring.

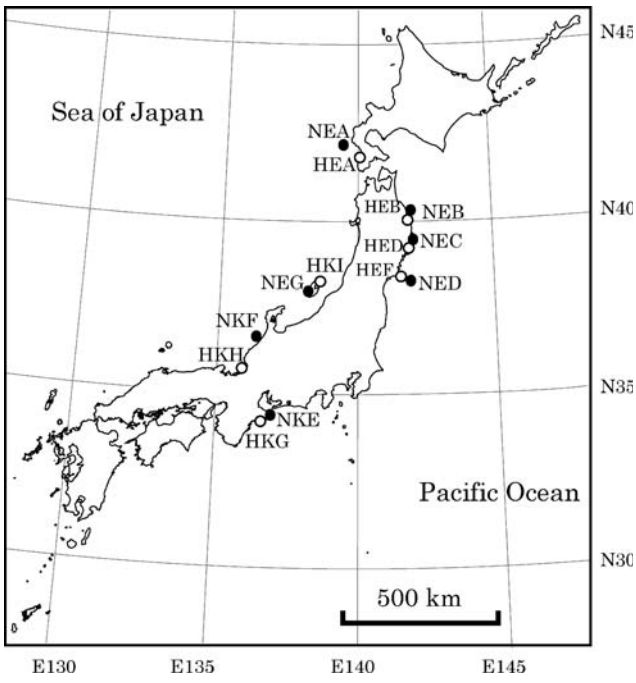

Fig. 1. Sampling sites of hatchery stocks (○) and natural populations (●).

Microsatellite Analyses. A DNA sample of each individual was extracted from a small part of shell foot muscles using the standard phenol/chloroform procedure (Sambrook et al., 1989). Nine microsatellite loci (*Hd527*– GenBank accession nos. AB178064; *Hd535* – AB178065; *Hd553* – AB178066; *Hd601* – AB178069; *Hd604* – AB178070; *Hd680* – AB178073; *Hd715* – AB178074; *Hd731* – AB178077; and *Ahdh1147* – AB17083) were amplified using previously reported primer pairs (Hara and Sekino 2005; Sekino and Hara, submitted). Polymerase chain reaction to amplify each microsatellite locus, and allele detection and designations were performed in the same manner as described in Hara and Sekino (2005).

Statistical Analysis. Genetic variability statistics within a population were calculated, which involved the number of alleles detected at each microsatellite locus (A); allelic richness (R_s), which is an estimate of the number of alleles at each locus in each population independent of the sample size (El-Mousadik and Petit, 1996); and expected heterozygosity (H_E), using the FSTAT version 2.9.3 (Goudet, 1995) and the CERVUS version 2.0 software package (Marshall et al., 1998). Test for Hardy-Weinberg's equilibrium (HWE) at each locus was carried out based on a test analogous to Fisher's exact test with the Markov-chain method (Markov-chain length, 100,000; dememorization, 10,000) using the ARLEQUIN version 3.0 package (Excoffier et al., 2005). Significance levels for all multiple tests were corrected following Rice (1989).

A multilocus estimate of genetic population heterogeneity for each pair of populations, F_{ST} value (Weir and Cockerham, 1984), was estimated, and the significance of pairwise F_{ST} values was evaluated through 10,000 permutation procedures. We also addressed the genetic relationship of population samples according to a genetic distance measure (modified Cavalli-Sforza chord distance: D_A, Nei et al., 1983). Unrooted neighbor-joining tree (NJ tree, Saito and Nei, 1987) based on the D_A, distance was constructed,

Table 1. Sampling Sites, Date of Sampling, Sampling Size, and Founding Records of Hatchery Stocks[a]

Sampling site (prefecture)	*Abbreviation*	*Date sampled*	*Sample size*	*Number of parents*	*Generation*	*Source of poplations*
Hatchery stocks						
Hakkaido, Kumaisi	HEA	Sept., 2003	96	10<	6–7	E
Iwate, Omoe	HEB	Apr., 2003	72	15<	F	E
Iwate, Omoe	HEC	Apr., 2003	72	15<	F–2	E
Iwate, Oofunato	HED	Apr., 1999	58	20>	F	E
Iwate, Kesen	HEE	Apr., 1999	72	10<	4–5	E
Miyagi, Yagawa	HEF	Apr., 2000	93	15<	F	E
Mie, Hamajima	HKG	Nov., 1999	62	20>	F	K
Fukui, Obama	HKH	Nov., 2003	60	20>	F	K
Niigata, Ryotsu	HKI	Nov., 2003	64	10<	F	K
Natural populations						
Hokkaido, Iwanai	NEA	Nov., 2000–Dec. 2002	103	–	–	E
Iwate, Omoe	NEB	Sept., 2004	60	–	–	E
Iwate, Hirota	NEC	Nov., 2000	96	–	–	E
Miyagi, Kinkasan	NED	Nov., 2001	96	–	–	E
Mie, Ijika	NKE	Aug., 1999	68	–	–	K
Fukui, Mikuni	NKF	Nov., 2000–Dec., 2002	72	–	–	K
Niigata, Ryotsu	NKG	May, 2004	63	–	–	K

[a]Founding record: Number of parents are actual number of abalone used as founders but not necessarily the effective number of parents.
Genereation: F is first generation and the number is generation number.
Source of population: E: Ezo-abalone origin, K: Kuro-abalone origin.

and the tree was evaluated calculating bootstrap values through 1000 resamplings across loci on the DISPAN program http://iubio.bio.indiana.edu:7780/perl/custom/index.cgidir=/public/molbio/genetic/pop/Dispan). The trees were visualized using the TREE VIEW program (Page, 1996).

The self-assignment test was performed to examine the potential of the microsatellite markers for discrimination among the hatchery stocks at the individual level without parental genotype information. Each hatchery stock was allocated to the most probable population of origin using the GeneClass2 version 2.0 software (Piry et al., 2004) with the distance-based assignment option, which can be used in broader situation without a HWE agreement and gametic phase equilibrium. We adopted the D_A distance to measure relatedness between individuals, and the probability of an individual belonging to a candidate population was calculated (Cornuet et al., 1999). The threshold probability value to reject the possibility that an individual belongs to a population was set at 0.01.

Results

Genetic Variability. Genetic variability indices for the seven natural populations and nine hatchery stocks in Ezo-abalone and Kuro-abalone are summarized in Table 2. The number of alleles per locus (*A*) and allelic richness per locus (*R*s) ranged from 4.5 to 11.3 and from 4.4 to 10.0, respectively. We compared *R*s values, rather than *A* values, between hatchery stocks and natural populations, as it is expected that the *A* value varies widely depending on the sample size of populations. The average of R_s values in the nine hatchery stocks (4.4-8.2) were substantially lower than those in the seven natural populations (9.5-11.3; Kruskal-Wallis test, df = 1, $H = 11.1$, $P < 0.001$), and consequently the hatchery stocks showed 36% reduction of the R_s values compared with those calculated for the natural populations. The R_s value was not significantly different between the Ezo-abalone and Kuro-abalone in both the natural populations (Kruskal-Wallis test, df = 1, $H = 0.5$, $P = 0.48$) and hatchery stocks (df = 1, $H = 3.2$, $P = 0.07$). A comparison of mean H_E values revealed that the values of the hatchery stocks (H_E: 0.497-0.648) were significantly lower than the values of the natural populations (H_E: 0.592-0.655; Kruskal-Wallis test, df = 1, $H = 5.7$, $P = 0.02$). As well as the case of the R_S, there was no significance regarding the difference of mean H_E values between the Ezo-abalone and Kuro-abalone in the natural populations (Kruskal-Wallis test, df = 1, $H = 0.0$, $P = 0.96$) and in the hatchery stocks (df = 1, $H = 1.1$, $P = 0.30$).

HWE testing revealed that 37 of 144 locus-population combinations were discordant with HWE at the uncorrected significance level ($P < 0.05$), and 17 combinations showed significant deviations from HWE after the Bonferroni correction (nine simultaneous tests, $P < 0.006$). Almost all the deviations were observed in the hatchery stocks

Table 2. Number of Alleles (A), Allelic Richness (R_s), Observed Heterozygosity (H_0), Expected Heterozygosity (H_E) at the Nine Microsatellite Loci Examined in 16 Pacific Abalone Samples

		Hatchery stocks															
Locus		HEA		HEB		HEC		HED		HEE		HEF		HKG		HKH	
Hd527	A/Rs	1	1.0	1	1.0	1	1.0	3	3.0	1	1.0	2	2.0	3	2.9	3	2.9
	Ho/He	0	0	0	0	0	0	0.224	0.203	0	0	0.043	0.042	0.068	0.066	0.067	0.065
	P	1.000		1.000		1.000		1.000		1.000		1.000		1.000		1.000	
Hd535	A/Rs	10	9.0	9	8.7	11	10.9	12	11.9	8	8.0	11	9.8	14	13.8	17	16.8
	Ho/He	0.781	0.728	0.944	0.823	0.903	0.862	0.897	0.847	0.778	0.807	0.904	0.837	0.852	0.884	0.883	0.902
	P	0.072		0.000		0.000		0.264		0.279		0.001		0.113		0.000	
Hd553	A/Rs	2	2.0	2	2.0	2	2.0	5	5.0	3	2.8	4	3.8	6	6.0	6	6.0
	Ho/He	0.521	0.397	0.222	0.199	0.569	0.443	0.724	0.505	0.352	0.295	0.564	0.474	0.613	0.643	0.867	0.688
	P	0.001		0.586		0.017		0.000		0.332		0.179		0.019		0.006	
Hd601	A/Rs	6	5.2	10	9.8	7	6.7	7	7.0	6	5.7	9	8.1	10	9.7	11	10.8
	Ho/He	0.531	0.482	0.736	0.764	0.736	0.700	0.638	0.647	0.653	0.696	0.819	0.752	0.672	0.631	0.683	0.747
	P	0.681		0.125		0.002		0.197		0.641		0.003		0.436		0.533	
Hd604	A/Rs	7	7.0	10	10.0	8	7.8	9	9.0	7	6.8	8	7.6	14	13.9	10	9.9
	Ho/He	0.583	0.844	0.903	0.853	0.819	0.806	0.750	0.800	0.775	0.711	0.772	0.770	0.852	0.904	0.847	0.831
	P	0.000		0.000		0.035		0.386		0.254		0.041		0.098		0.005	
Hd680	A/Rs	2	2.0	5	5.0	4	4.0	4	4.0	2	2.0	4	4.0	6	5.9	5	5.0
	Ho/He	0.229	0.235	0.736	0.678	0.778	0.648	0.483	0.611	0.444	0.404	0.624	0.635	0.242	0.316	0.569	0.472
	P	0.674		0.067		0.000		0.075		0.556		0.241		0.000		0.203	
Hd715	A/Rs	3	3.0	4	4.0	5	5.0	8	7.9	5	4.8	5	4.6	7	6.9	8	7.9
	Ho/He	0.417	0.381	0.708	0.672	0.542	0.639	0.862	0.754	0.625	0.618	0.609	0.527	0.516	0.528	0.600	0.556
	P	0.401		0.012		0.028		0.342		0.999		0.371		0.799		0.838	
Hd731	A/Rs	6	6.0	8	7.6	4	4.0	7	7.0	4	4.0	6	6.0	3	3.0	6	5.9
	Ho/He	0.842	0.757	0.806	0.797	0.750	0.678	0.679	0.726	0.667	0.623	0.833	0.750	0.607	0.598	0.414	0.451
	P	0.009		0.046		0.303		0.062		0.756		0.000		0.055		0.029	
Ahdh1147	A/Rs	4	4.0	3	3.0	4	4.0	4	4.0	4	4.0	4	3.9	3	3.0	4	3.9
	Ho/He	0.677	0.653	0.667	0.659	0.694	0.618	0.714	0.736	0.889	0.723	0.634	0.607	0.210	0.269	0.661	0.655
	P	0.475		0.194		0.016		0.829		0.070		0.290		0.000		0.070	
Mean	A/Rs	4.6	4.4	6.1	6.0	5.3	5.2	6.9	6.8	4.5	4.4	6.1	5.7	7.9	7.8	8.3	8.2
	Ho/He	0.509	0.497	0.636	0.605	0.643	0.599	0.663	0.648	0.576	0.542	0.645	0.599	0.515	0.538	0.621	0.596

		Natural populations													
HK		NEA		NEB		NEC		NED		NKE		NKF		NKG	
2	2.0	4	3.9	5	4.8	6	4.7	5	4.7	3	2.8	4	4.0	3	3.0
0.063	0.061	0.118	0.114	0.100	0.097	0.104	0.130	0.146	0.159	0.147	0.163	0.153	0.170	0.175	0.163
1.000		1.000		–		0.049		0.216		0.426		0.026		1.000	
9	9.0	21	17.1	16	15.9	21	18.3	17	15.2	20	19.1	20	18.6	15	14.8
0.906	0.856	0.893	0.903	0.915	0.913	0.917	0.918	0.863	0.899	0.868	0.922	0.944	0.908	0.871	0.907
0.001		0.009		0.157		0.024		0.813		0.587		0.906		0.405	
6	5.8	5	4.3	5	4.9	6	5.1	7	6.4	5	5.0	5	5.0	8	7.9
0.766	0.694	0.233	0.247	0.450	0.398	0.396	0.418	0.417	0.463	0.632	0.673	0.775	0.681	0.823	0.720
0.013		0.394		0.854		0.186		0.176		0.403		0.114		0.306	
8	7.9	18	15.5	14	13.7	17	15.1	17	14.6	16	15.0	16	14.8	13	13.0
0.734	0.671	0.660	0.641	0.683	0.701	0.740	0.743	0.635	0.682	0.838	0.795	0.718	0.759	0.857	0.832
0.089		0.961		0.113		0.790		0.596		0.276		0.152		0.770	
9	8.9	13	11.8	14	13.9	14	13.4	13	12.3	13	12.9	11	11.0	16	15.7
0.906	0.746	0.835	0.835	0.783	0.878	0.698	0.876	0.813	0.878	0.868	0.896	0.831	0.885	0.787	0.889
0.000		0.943		0.192		0.000		0.355		0.867		0.098		0.017	
4	4.0	6	4.6	5	4.9	7	6.0	7	5.8	7	6.5	7	6.6	7	6.9
0.422	0.500	0.524	0.598	0.650	0.601	0.625	0.620	0.479	0.569	0.485	0.525	0.333	0.351	0.393	0.464
0.289		0.352		0.015		0.824		0.462		0.415		0.093		0.086	
5	5.0	9	8.7	8	7.9	9	8.5	9	8.8	9	8.8	7	6.8	8	8.0
0.609	0.560	0.544	0.560	0.567	0.612	0.594	0.629	0.625	0.591	0.544	0.547	0.528	0.530	0.508	0.580
0.774		0.166		0.196		0.125		0.738		0.306		0.357		0.075	
4	3.9	8	7.5	7	7.0	10	9.0	9	8.7	5	5.0	6	5.6	6	6.0
0.714	0.567	0.657	0.725	0.724	0.778	0.833	0.788	0.787	0.811	0.559	0.665	0.625	0.641	0.557	0.662
0.032		0.234		0.450		0.351		0.200		0.235		0.085		0.030	
3	3.0	5	4.8	5	5.0	4	4.0	7	5.8	3	3.0	5	4.6	7	6.8
0.313	0.294	0.786	0.709	0.845	0.737	0.737	0.721	0.719	0.723	0.294	0.338	0.681	0.676	0.710	0.675
1.000		0.339		0.432		0.557		0.749		0.294		0.645		0.091	
5.9	5.8	10.5	9.2	9.3	9.1	11.3	10.0	10.5	9.6	9.8	9.4	9.5	9.0	9.5	9.4
0.604	0.550	0.583	0.592	0.635	0.635	0.627	0.649	0.609	0.642	0.582	0.614	0.621	0.622	0.631	0.655

P; exact Pvalue by the Markov chain method. Wide significance levels were applied using the sequential Bonferroni correction, k = 9, $P < 0.006$.

except one instance (IWH, natural population) and 13 of 16 cases indicated heterozygote excess.

Genetic Relationships of Population Samples. Multilocus estimates of genetic population heterogeneity, F_{ST}, were calculated to assess the genetic difference between 16 abalone popula tions (Table 3). Combinations of the 16 populations consistently yielded highly significant F_{ST} values (0.001–0.259) for almost all cases ($P < 0.0001$) except for three combinations of natural populations (NEA-NEC, NEB-NED, and NKF-NKG), between which the geographical distances were relatively small. The D_A genetic distance was calculated for all possible pairs of the 16 samples (Table 3). The D_A values between the hatchery stocks (D_A = 0.116–0.319) were obviously larger than those between the natural populations (D_A = 0.021–0.148, Kruskal-Wallis test, df = 1, H = 33.1, $P < 0.001$). The D_A between each hatchery stock and the geographically proximal natural population (D_A, 0.066–0.155; mean ± SD, 0.108 ± 0.035) showed smaller distances than that between the hatchery stocks (df = 1, H = 15.1, $P < 0.001$), but showed larger distances than that between the natural populations (df = 1, H = 3.9, P = 0.049). The NJ tree constructed on the basis of the D_A distances failed to allocate the 16 samples into any clear cluster. Almost all hatchery stocks were located at the tip of the tree with long branch lengths, and the natural populations were relatively congregated in the central part of the tree (Figure 2). This tree topology is consistent with a higher level of genetic divergence among the hatchery stocks compared to that among natural populations.

The results of individual assignment test also evidenced the high genetic divergence among the hatchery stocks (Table 4). This distance-based assignment method achieved a high success rate of individual assignment in the all hatchery stocks as 86% to 100% of individuals of each stock were correctly assigned to their origin. Also, relatively low proportions (3% to 12%) were rejected from its own stocks at the significant level ($P < 0.01$), furthermore 27 of all (4.9%) were rejected from the all hatchery stocks. These suggest the efficiency of the nine markers for discrimination among hatchery stocks.

Discussion

Substantial loss of allelic diversity was observed in all hatchery stocks compared to the natural populations, while little difference was found between Ezo- and Kuro-abalone hatchery stocks. Reduced allelic variabilities of microsatellites in hatchery-produced animals were reported in many species including the Pacific abalone (Coughlan et al., 1998, Was and Wenne, 2002; Li et al., 2004), which are attributable to population bottlenecks due to the small number of effective parents in most cases (Norris et al., 1999; Sekino et al., 2002). This is the case of hatchery stocks screened in this study, as six of the nine hatchery stocks were founded by less than 10 or15 candidate parents, although it is not possible to estimate the number of effective males and females. Consistent with the limited number of founders, the estimates of overall expected heterozygosity (H_E) in hatchery stocks were lower than those in natural populations. However, our results are inconsistent with previous findings in an allozyme survey, in which the allelic diversity of Ezo-abalone hatchery stocks was significantly decreased with little reduction of heterozygosity (Kijima et al., 1992). This incongruence between the allozyme and microsatellite analysis is simply ascribable to the sensibility of microsatellites in detecting reductions of genetic diversity (Li et al., 2004). The HEA and HEE stocks showed the lowest level of genetic variations in terms of both mean heterozygosity and R_S value. Because the two were created by founders maintained over four generations in the hatcheries, inbreeding as well as population bottlenecks would have precipitated the decay of genetic diversity in these stocks.

Significant deviations from HWE were observed in hatchery stocks but not in almost all the natural populations except for HEE stock. Thirteen of the 16 significant HWE deviations in hatchery stocks revealed heterozygote excess, and Li et al. (2004) showed, based on microsatellite analysis, that heterozygote excesses were observed in 8 of 10 significant HWE deviations in Ezo-abalone hatchery stocks. Population bottlenecks sometimes give rise to a reduction of homozygotes when the population of interest experienced a recent reduction in size, owing to sampling bias of alleles resulted from a small number of parents and differences in allele frequencies between the sexes (Spencer et al., 2000; Launey et al., 2001 and references therein). Alternatively, it is possible to ascribe a heterozygote excess in cultured stocks to overdominance phenomenon, which causes a lower survival of homozygotes (Sugita and Fujio, 1982; Fujio et al., 1985). This study was not designed to determine the cause of the heterozygote excesses; controlled crossbreeding experiments may uncover an underlying overdominance in Ezo-abalone (e.g., Launey and Hedgecock, 2001).

The high genetic distances (F_{ST} and D_A) estimated for between the hatchery stocks provided evidence of substantial genetic divergence of the hatchery stocks, consistent with a population bot-

Table 3. Genetic Differentiation Between Pairwise Samples Using Estimate F_{ST} of (Above Diagonal) and D_A Genetic Distance (Below Diagram)

	HEA	HEB	HEC	HED	HEE	HEF	HKG	HKH	HKI	NEA	NEB	NEC	NED	NKE	NKF	NKG
HEA	–	0.150* (0.0000)	0.145* (0.0000)	0.120* (0.0000)	0.206* (0.0000)	0.105* (0.0000)	0.259* (0.0000)	0.183* (0.0000)	0.197* (0.0000)	0.065* (0.0000)	0.098* (0.0000)	0.075* (0.0000)	0.076* (0.0000)	0.194* (0.0000)	0.175* (0.0000)	0.171* (0.0000)
HEB	0.229	–	0.056* (0.0000)	0.070* (0.0000)	0.110* (0.0000)	0.062* (0.0000)	0.156* (0.0000)	0.080* (0.0000)	0.134* (0.0000)	0.066* (0.0000)	0.058* (0.0000)	0.044* (0.0000)	0.047* (0.0000)	0.102* (0.0000)	0.080* (0.0000)	0.070* (0.0000)
HEC	0.218	0.116	–	0.069* (0.0000)	0.137* (0.0000)	0.071* (0.0000)	0.109* (0.0000)	0.074* (0.0000)	0.122* (0.0000)	0.080* (0.0000)	0.057* (0.0000)	0.049* (0.0000)	0.053* (0.0000)	0.065* (0.0000)	0.058* (0.0000)	0.058* (0.0000)
HED	0.177	0.175	0.171	–	0.094* (0.0000)	0.059* (0.0000)	0.128* (0.0000)	0.095* (0.0000)	0.129* (0.0000)	0.037* (0.0000)	0.030* (0.0000)	0.021* (0.0000)	0.024* (0.0000)	0.092* (0.0000)	0.066* (0.0000)	0.068* (0.0000)
HEE	0.250	0.204	0.223	0.181	–	0.123* (0.0000)	0.203* (0.0000)	0.161* (0.0000)	0.207* (0.0000)	0.105* (0.0000)	0.095* (0.0000)	0.081* (0.0000)	0.091* (0.0000)	0.151* (0.0000)	0.130* (0.0000)	0.129* (0.0000)
HEF	0.182	0.132	0.145	0.141	0.190	–	0.141* (0.0000)	0.088* (0.0000)	0.152* (0.0000)	0.034* (0.0000)	0.040* (0.0000)	0.020* (0.0000)	0.025* (0.0000)	0.075* (0.0000)	0.072* (0.0000)	0.079* (0.0000)
HKG	0.319	0.211	0.197	0.214	0.298	0.201	–	0.094* (0.0000)	0.157* (0.0000)	0.171* (0.0000)	0.117* (0.0000)	0.118* (0.0000)	0.110* (0.0000)	0.022* (0.0000)	0.056* (0.0000)	0.058* (0.0000)
HKH	0.285	0.147	0.148	0.196	0.230	0.143	0.141	–	0.063* (0.0000)	0.109* (0.0000)	0.076* (0.0000)	0.071* (0.0000)	0.057* (0.0000)	0.055* (0.0000)	0.023* (0.0000)	0.018* (0.0000)
HKI	0.261	0.228	0.205	0.232	0.293	0.209	0.196	0.130	–	0.159* (0.0000)	0.115* (0.0000)	0.113* (0.0000)	0.104* (0.0000)	0.116* (0.0000)	0.066* (0.0000)	0.054* (0.0000)
NEA	0.120	0.127	0.155	0.078	0.165	0.093	0.208	0.156	0.206	–	0.024* (0.0000)	0.006 (0.002)	0.011* (0.0000)	0.114* (0.0000)	0.089* (0.0000)	0.094* (0.0000)
NEB	0.174	0.136	0.150	0.099	0.177	0.112	0.153	0.134	0.181	0.059	–	0.009* (0.0000)	0.010* (0.0000)	0.065* (0.0000)	0.056* (0.0000)	0.055* (0.0000)
NEC	0.142	0.105	0.128	0.075	0.155	0.077	0.160	0.115	0.172	0.026	0.046	–	0.001 (0.361)	0.067* (0.0000)	0.051* (0.0000)	0.052* (0.0000)
NED	0.148	0.123	0.152	0.081	0.160	0.087	0.145	0.107	0.167	0.032	0.046	0.021	–	0.061* (0.0000)	0.045* (0.0000)	0.045* (0.0000)
NKE	0.283	0.166	0.142	0.181	0.236	0.129	0.072	0.086	0.152	0.148	0.109	0.097	0.092	–	0.034* (0.0000)	0.032* (0.0000)
NKF	0.254	0.137	0.113	0.144	0.209	0.126	0.098	0.066	0.115	0.121	0.099	0.083	0.083	0.055	–	0.001 (0.298)
NKG	0.258	0.136	0.133	0.154	0.218	0.143	0.094	0.070	0.114	0.124	0.099	0.082	0.076	0.055	0.032	–

Wide significance levels were applied using the sequential Bonferroni correction, k = 120, * = $P < 0.0004$.

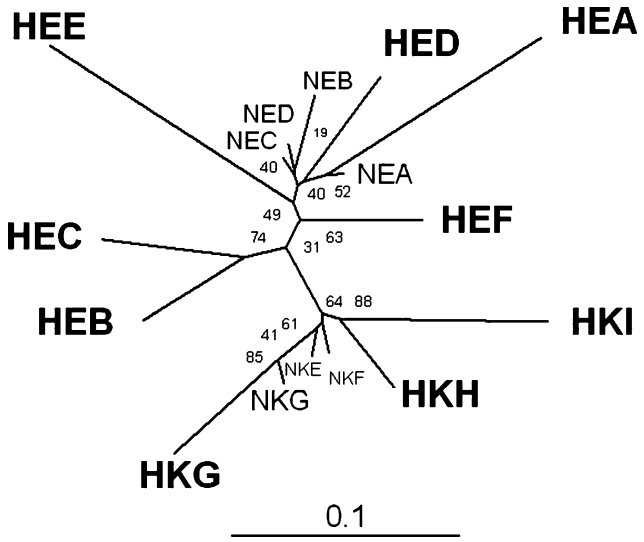

Fig. 2. A unrooted neighbor–joining tree generated from modified Cavalli–Sforza chord distance (D_A) for the nine hatchery strains and the six natural populations. The numbers refer to percentage bootstrap values generated from 1000 replications of resampled loci.

tleneck and the subsequent genetic drift. The high rate of assignment success for individuals from the hatchery stocks (86% to 100%) is also accounted for by the high genetic differentiation among the hatchery stocks because the success rate of individual assignment test depends largely on the magnitude of genetic differences among populations, from which individuals to be tested originate (Cornuet et al., 1999). These could be the result that the hatchery stocks are characterized with not only low genetic diversity but also heterogeneous allelic composition. It should be noted that based on the D_A distance the extent of genetic differentiation between each hatchery stock and its geographically proximal natural population (i.e., the population closely related to the source population of each stock) is similar to those observed between natural Ezo-abalone (cold-current type) and Kuro-abalone (warm-current type) populations (D_A, 0.083–0.148; mean ± SD, 0.101 ± 0.021). Nonetheless, there remains taxonomic uncertainty between Ezo- and Kuro-abalone; it is expected that they adapt biologically and genetically to the environmental condition of each habitat (Ino, 1952) and should be delineated genetically as separate management units (Hara and Sekino, 2005). If such hatchery stocks with genetic characteristics highly divergent from that of natural populations achieve a high reproductive success after the release, the stocking practices would be at risk of having a great genetic impact on natural abalone resources. Thus, it is necessary to find an allowable range of amount of

Table 4. Result of Self-Assignment Test of the Nine Hatchery Stocks Using the D_A Distance Method

Rate(%)	HEA	HEB	HEC	HED	HEE	HEF	HKG	HKH	HKI	Reject from all
HEA (n = 96)										
Assign to	**99.0**	0	0	1.0	0	0	0	0	0	
Reject from	**3.1**	90.6	97.9	43.8	100.0	65.6	99.0	86.5	96.9	1.0
HEB(n = 72)										
Assign to	0.0	**90.3**	5.6	1.4	1.4	1.4	0.0	0.0	0.0	
Reject from	97.2	**8.3**	62.5	80.6	87.5	63.9	93.1	59.7	98.6	2.8
HEC(n = 72)										
Assign to	0	2.8	**91.7**	1.4	0	1.4	0	2.8	0.0	
Reject from	100.0	44.4	**5.6**	66.7	100.0	62.5	81.9	40.3	95.8	2.8
HED(n = 58)										
Assign to	1.7	0	1.7	**94.8**	0	0	0	0	0	
Reject from	100.0	94.8	94.8	**6.9**	98.3	81.0	93.1	89.7	100.0	6.9
HEE(n = 72)										
Assign to	0	0	0	0	**100.0**	0	0	0	0	
Reject from	100.0	83.3	81.9	50.0	**4.2**	75.0	98.6	75.0	100.0	2.8
HEF(n = 93)										
Assign to	4.3	4.3	1.1	1.1	0	**86.0**	3.2	0.0	0.0	
Reject from	94.6	66.7	82.8	49.5	94.6	**6.5**	90.3	29.0	97.8	2.2
HKG(n = 62)										
Assign to	0	0	0	0	0	0	**95.2**	4.8	0.0	
Reject from	100.0	93.5	93.5	75.8	100.0	95.2	**9.7**	33.9	96.8	9.7
HKH(n = 60)										
Assign to	0	1.7	1.7	0	0	0	0	**90.0**	0.0	
Reject from	100.0	78.3	91.7	96.7	100.0	85.0	85.0	**11.7**	81.7	8.3
HKI(n = 64)										
Assign to	0	0	0	0	0	0	3.1	0	**96.9**	
Reject from	100.0	98.4	90.6	90.6	100.0	90.6	70.3	15.6	**6.3**	4.7

Note: The numbers in bold denote the proportion of individuals assigned to or rejected from their strain of origin.

release within which the alteration of genetic makeup in natural populations is minimized and the natural biomass is maximized. Answering this question requires the knowledge of reproductive contribution of released hatchery-produced abalone to the natural resources. The top priority of our future study should therefore be given to estimate the extent of reproductive success of released abalone under the natural environment, which can be addressed by collecting microsatellite genotype information of ensuing generations.

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