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Population genetic structure of wild and hatchery black rockfish *Sebastes inermis* in Korea, assessed using cross-species microsatellite markers

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ABSTRACT. The population structure of the black rockfish, *Sebastes inermis* (Sebastidae), was estimated using 10 microsatellite loci developed for *S. schlegeli* on samples of 174 individuals collected from three wild and three hatchery populations in Korea. Reduced genetic variation was detected in hatchery strains [overall number of alleles (N_A) = 8.07; allelic richness (A_R) = 7.37; observed heterozygosity (H_O) = 0.641] compared with the wild samples (overall N_A = 8.43; A_R = 7.83; H_O = 0.670), but the difference was not significant. Genetic differentiation among the populations was significant (overall F_{ST} = 0.0237, P < 0.05). Pairwise F_{ST} tests, neighbor-joining tree, and

principal component analyses showed significant genetic heterogeneity among the hatchery strains and between wild and hatchery strains, but not among the wild populations, indicating high levels of gene flow along the southern coast of Korea, even though the black rockfish is a benthic, non-migratory marine species. Genetic differentiation among the hatchery strains could reflect genetic drift due to intensive breeding practices. Thus, in the interests of optimal resource management, genetic variation should be monitored and inbreeding controlled within stocks in commercial breeding programs. Information on genetic population structure based on cross-species microsatellite markers can aid in the proper management of *S. inermis* populations.

Key words: Black rockfish; *Sebastes inermis*; Genetic structure; Hatchery strain; Microsatellite loci; Wild population

INTRODUCTION

Rockfishes (genus *Sebastes*) are important members of the near-shore demersal community in both the Pacific and the Atlantic oceans, with approximately 105 species found worldwide (Hyde and Vetter, 2007). Among them, the Korean black rockfish, *Sebastes inermis*, is distributed in coastal waters from southern Hokkaido to Kyushu, Japan, and the southern coasts of the Korean Peninsula (Utaga and Taniuchi, 1999), inhabiting rocky reefs and feeding on small fishes and marine invertebrates (Nakabo, 2000). This species is an important commercial fishery resource in Korea and has long been considered a common fishery resource of the southern coast of Korea. However, annual catches have declined drastically since the late 1990s. Reasons for the decline remain unknown, although habitat loss resulting from coastal area development, eutrophication, and overfishing may be contributing factors. The decline of Korean black rockfish has fostered increased interest in the potential for aquaculture or supplementation of natural populations. Thus, artificial reproduction has been practiced since 2000, and large numbers of cultured fry have been released into southern Korean coastal waters since 2003.

As a consequence of the rapid increase in hatchery-reared black rockfish production, the genetic composition of natural black rockfish populations must be understood to evaluate potential genetic effects as a result of hatchery operations. Recently, conservationists have focused on the extent to which stocking impacts indigenous populations. The genetic diversity of artificial seeds is generally lower than that of wild populations, due to unconscious selection and the limited number of parents used to produce subsequent generations in the hatchery (Coughlan et al., 1998; Sekino et al., 2002; Skaala et al., 2004). Reduced genetic diversity observed in most hatchery stocks could result in a loss of genetic variation, reducing the ability of the population to adapt to new environments (Allendorf and Phelps, 1980; Liu et al., 2005). Thus, understanding patterns of stock structure is a prerequisite for successful aquaculture management and preservation of aquatic biodiversity in the sustainable development of marine fisheries (Reiss et al., 2009). However, despite its importance to the fishery industry in Korea, little is known about the genetic diversity and population structure of *S. inermis*.

Microsatellite (MS) DNA markers are a useful tool for evaluating the genetic diversity

and structure of various species, including fish. Although marine organisms have been thought to constitute homogeneous entities, MS studies have revealed the presence of subtle genetic structures at small and large geographic scales (Knutsen et al., 2003; Nielsen et al., 2003; Jørgensen et al., 2005).

The aim of this study was to investigate the genetic diversity of wild and hatchery-reared Korean *S. inermis* population samples to determine whether fish culture practices have reduced genetic variation and to assess the population structure of these samples.

MATERIAL AND METHODS

Sample collection and DNA extraction

For MS analysis, in total, 174 individuals of *S. inermis* were collected from three localities on the southern coast of Korea that were assigned to the six populations in 2007 and 2008 (Figure 1). The three wild populations were collected from the fish landing and auction places in Yeosu [Y (N = 30)], Tongyeong [T (N = 20)], and Namhae [N (N = 30)]. The three hatchery populations were collected from cage culture farms in Yeosu [YH (N = 32)], Tongyeong [TH (N = 32)], and Namhae [NH (N = 30)]. Although the hatchery populations had been reared continuously, details of their origins and records were unavailable (Table 1).

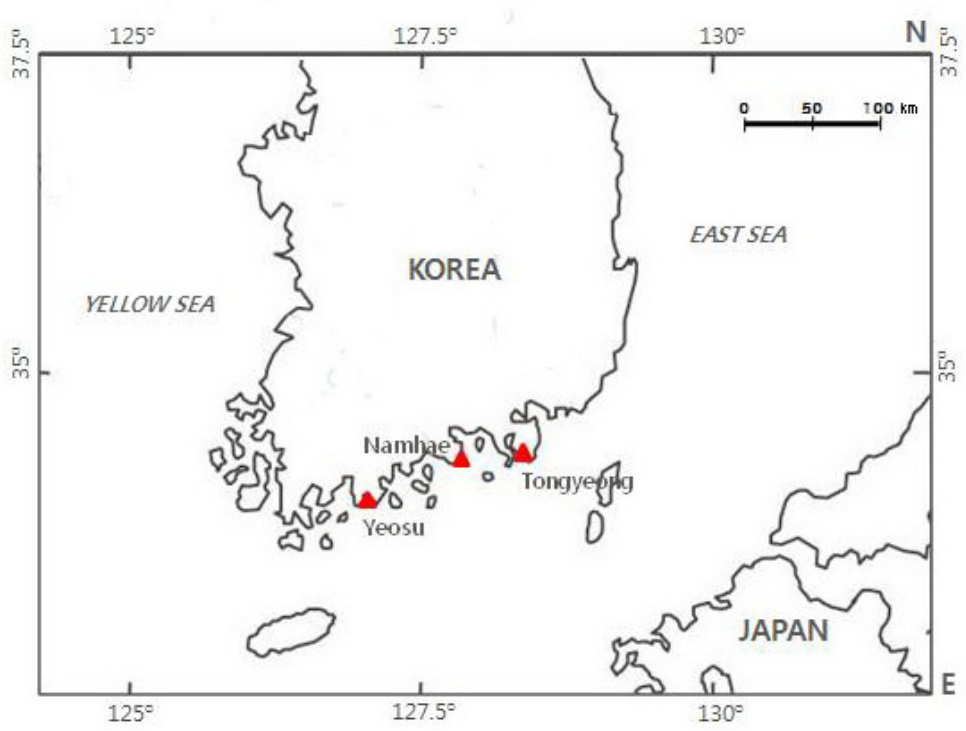

Figure 1. Sampling sites of wild and hatchery populations of *Sebastes inermis*.

Table 1. Collection details for black rockfish *Sebastes inermis* samples.

Sampling area (abbreviation)	Sample locality	Sample size	Collection date
Wild population			
Yeosu (Y)	South Sea; 34° 36' N, 127° 43' E	30	May 2007
Tongyeong (T)	South Sea; 34° 50' N, 128° 16' E	20	June 2007
Namhae (N)	South Sea; 34° 49' N, 127° 56' E	30	June 2007
Hatchery cultured strains			
Yeosu (YH)	South Sea; 34° 36' N, 127° 45' E	32	October 2007
Tongyeong (TH)	South Sea; 34° 44' N, 128° 20' E	32	December 2007
Namhae (NH)	South Sea; 34° 48' N, 127° 57' E	30	December 2007

Fin clips were preserved in 99.9% ethanol before being transported to the laboratory. Total DNA was extracted using a MagExtractor-Genomic DNA Purification Kit (Toyobo, Japan), according to the manufacturer protocol for the automated DNA extraction system MagExtractor MFX-2100 (Toyobo). Extracted genomic DNA was stored at -20°C until polymerase chain reaction (PCR) analysis.

Microsatellite genotyping

In total, 174 Korean black rockfish from the six populations were genotyped. Ten microsatellite loci (KSs2A, KSs3, KSs5, KSs6, KSs8, KSs11B, KSs16, KSs18A, KSs20A, and KSs27A; GenBank accession Nos. EF109802-7, EF109809, EF109811, EF109813, and EF109815) were amplified using PCR, with the primers and protocols developed previously for *S. schlegeli* by An et al. (2009).

The 5'-end of the forward primer of each set of primers was labeled with fluorescent dye (6-FAM, HEX, or NED; Applied Biosystems, USA). PCR amplification of the 10 MS loci was carried out in 10-µL volumes containing 0.25 U Taq DNA polymerase, 10X ExTaq buffer, 2 mM dNTP mixture (Takara, Japan), 2 µM each primer set and about 10-50 ng template DNA using a PTC-0220 DNA Engine Dyad Peltier thermal cycler (MJ Research, Inc., 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 at each primer temperature listed in An et al. (2009) for 1 min, 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 of each reaction containing formamide (Hi-Di Formamide, Applied Biosystems, UK) and GeneScan® 400HD [ROX] size standard (ABI PRISM, Applied Biosystems, USA), denatured at 95°C for 2 min, and immediately chilled on ice. Fragment analysis of the reaction product was carried out using an ABI 3130 Genetic Analyzer (Applied Biosystems) and the GENEMAPPER software (version 4.0; Applied Biosystems).

Data analysis

Statistical genetic analyses were conducted on six populations of *S. inermis*. PCR errors as a result of null alleles, stuttering, or allele dropout were tested using MICRO-CHECKER (ver. 2.2.3; 1000 randomizations; van Oosterhout et al., 2004). Scoring and human error were estimated by duplicate analyses. As genetic diversity parameters, the number of alleles per locus (N_A) and number of unique alleles (U) were determined for each local sample at each locus,

using the GENEPOP program (version 4.0; http://kimura.univ-montp2.fr/~rousset/Genepop.htm). Allelic richness (A_R) was corrected to the smallest sample (N = 20), using the rarefaction method of FSTAT (version 2.9.3.2; Goudet, 1995). A_R can be directly compared among populations, regardless of sample size (El Mousadik and Petit, 1996). For analysis of molecular variance (AMOVA; Excoffier et al., 1992), components of variance within and between populations based on the infinite allele model were estimated using the ARLEQUIN 3.0 program (Excoffier et al., 2005). The significance of AMOVA components was tested using 1000 permutations. To estimate genetic heterozygosity among the entire set of pairwise population samples, unbiased expected and observed heterozygosity values (H_E and H_O, respectively; Nei, 1987) were calculated. Deviations from Hardy-Weinberg equilibrium (HWE) for each locus and globally across populations and loci were tested using probability tests or exact tests of the Markov-chain procedure of ARLEQUIN.

The extent of population subdivision was examined by calculating global multilocus F_{ST} values (1000 permutations; Weir and Cockerham, 1984) using ARLEQUIN. Significance levels were adjusted for multiple tests using the sequential Bonferroni correction technique (Rice, 1989).

The genetic distance between populations was estimated based on the chord distances (D_{CE}; Cavalli-Sforza and Edwards, 1967). A phylogenetic tree was constructed based on pairwise genetic distances for all samples using the neighbor-joining (NJ) method of the POPULATION program (version 1.2.30), to examine genetic relationships between populations (Langella, 2007). Bootstrap values were calculated using 1000 replicates. The NJ tree (Saito and Nei, 1987) was visualized using the Tree Explorer program.

Relationships among geographical populations were assessed using principal component analysis (PCA), based on the covariance matrix of gene frequencies using GenAlEx 6.3 (http://www.anu.edu.au/BoZo/GenAlEx/).

RESULTS

Genetic variability

MICRO-CHECKER analysis revealed that five of the 10 loci (KSs2A, KSs3, KSs6, KSs11B, and KSs18A) could be affected by null alleles. However, we used these loci in this study because no null alleles affected all populations, and we found no indication of allele scoring error caused by stuttering or large allele dropout.

In total, 128 different alleles were observed across all loci for all samples (Table 2). No population had a diagnostic allele. The average number of alleles per locus was 8.25, ranging from 4.8 at KSs20A to 15 at KSs2A, across all populations, with the degree of variability differing considerably among the 10 loci. The mean of H_E and H_O per locus ranged from 0.518 at KSs5 to 0.891 at KSs6 and from 0.500 at KSs5 to 0.742 at KSs3, respectively. These results suggest that all MS loci were polymorphic, with large differences in the number of alleles and some level of polymorphism in all *S. inermis* populations studied.

Variable differences in the number of alleles among populations were observed at all of these loci (Table 2). The mean N_A in wild population samples ranged from 8.3 to 8.6 and from 7.4 to 9.1 in hatchery populations. Additionally, the mean A_R of wild populations ranged from 7.52 to 8.30 and from 6.85 to 8.25 in hatchery strains. The mean H_O values estimated

Table 2. Allelic variability observed at 10 microsatellite loci in three wild and three hatchery *Sebastes inermis* populations of southern Korea.

Population	Microsatellite loci										
	KSs2A	KSs3	KSs5	KSs6	KSs7	KSs11B	KSs16	KSs18A	KSs20A	KSs27A	Mean
Wild populations											
Y (N = 30)											
N_A (U)	19 (2)	10 (0)	5 (0)	14 (0)	6 (1)	8 (0)	6 (0)	7 (1)	4 (0)	5 (1)	8.4 (0.5)
A_R	16.245	9.287	4.782	12.253	5.890	6.766	5.785	6.243	3.957	3.967	7.52
R	60	30	8	40	14	8	10	18	8	18	
H_E	0.904	0.871	0.559	0.910	0.727	0.453	0.779	0.646	0.673	0.792	0.731
H_O	0.850	0.650	0.504	0.969	0.750	0.250	0.750	0.600	0.600	0.750	0.667
P	NS	***	NS	NS	NS	**	NS	NS	NS	NS	
T (N = 20)											
N_A (U)	14 (0)	11 (1)	4 (3)	15 (1)	4 (0)	8 (0)	8 (0)	8 (0)	4 (0)	7 (0)	8.3 (0.4)
A_R	14	11	4	15	4	8	8	8	4	7	8.30
R	40	36	20	46	14	14	14	16	10	14	
H_E	0.905	0.880	0.552	0.891	0.758	0.711	0.734	0.711	0.538	0.586	0.727
H_O	0.867	0.667	0.500	0.784	0.900	0.567	0.713	0.600	0.500	0.500	0.660
P	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	
N (N = 30)											
N_A (U)	16 (0)	11 (0)	5 (0)	14 (0)	6 (0)	8 (2)	9 (0)	9 (1)	4 (1)	4 (1)	8.6 (0.5)
A_R	12.965	10.446	4.630	12.588	5.396	7.190	8.203	8.299	3.893	2.999	7.66
R	58	32	8	34	14	18	18	16	8	18	
H_E	0.725	0.863	0.467	0.885	0.733	0.806	0.767	0.737	0.619	0.623	0.722
H_O	0.700	0.900	0.433	0.600	0.833	0.687	0.667	0.833	0.533	0.633	0.682
P	NS	NS	NS	***	NS	**	NS	NS	NS	NS	
Hatchery strains											
YH (N = 32)											
N_A (U)	18 (0)	11 (0)	5 (0)	11 (2)	7 (0)	9 (0)	8 (1)	10 (0)	6 (0)	6 (0)	9.1 (0.3)
A_R	15.703	9.448	4.591	10.779	6.573	7.926	7.059	9.246	5.720	5.475	8.25
R	52	32	8	20	14	16	16	20	10	14	
H_E	0.895	0.861	0.444	0.894	0.666	0.491	0.770	0.790	0.626	0.799	0.724
H_O	0.756	0.750	0.531	0.633	0.756	0.375	0.724	0.563	0.750	0.770	0.661
P	***	***	NS	***	NS	***	NS	NS	NS	NS	

Continued on next page

Table 2. Continued.

Population	Microsatellite loci										
	KSs2A	KSs3	KSs5	KSs6	KSs7	KSs11B	KSs16	KSs18A	KSs20A	KSs27A	Mean
TH (N = 32)											
N_A (U)	11 (1)	10 (0)	7 (1)	11 (1)	5 (0)	6 (0)	5 (0)	6 (0)	6 (0)	7 (0)	7.4 (0.3)
A_R	9.306	9.148	5.824	11.000	4.606	5.846	4.983	5.992	5.603	6.201	6.85
R	32	30	20	24	14	14	10	16	10	16	
H_E	0.683	0.823	0.490	0.868	0.653	0.773	0.759	0.771	0.758	0.698	0.728
H_O	0.414	0.688	0.500	0.550	0.625	0.781	0.813	0.750	0.650	0.531	0.630
P	***	NS	NS	***	NS	NS	NS	NS	NS	NS	
NH (N = 30)											
N_A (U)	14 (0)	9 (0)	4 (0)	11 (0)	5 (0)	8 (1)	8 (0)	7 (0)	5 (0)	6 (0)	7.7 (0.1)
A_R	11.635	8.586	3.526	10.519	4.678	6.883	7.620	6.556	4.439	5.600	7.00
R	58	30	10	24	16	16	18	16	8	14	
H_E	0.807	0.880	0.599	0.895	0.593	0.705	0.744	0.697	0.699	0.605	0.722
H_O	0.700	0.800	0.533	0.667	0.567	0.667	0.700	0.633	0.567	0.500	0.633
P	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	
All populations											
N_A	15	10.3	5.0	13	5.5	7.8	7.3	7.8	4.8	5.8	8.25
H_E	0.820	0.863	0.518	0.891	0.688	0.656	0.759	0.725	0.652	0.684	
H_O	0.714	0.742	0.500	0.700	0.739	0.554	0.728	0.663	0.600	0.614	

Number of samples (N), number of alleles per locus (N_A), allellic richness (A_R), allellic size range (R), number of unique alleles (U), expected heterozygosity (H_E), observed heterozygosity (H_O), and probability of significant deviation from Hardy-Weinberg equilibrium (P) are given for each population and locus. NS = not significant; **P < 0.01; ***P < 0.001. Calculations assume that individuals with one microsatellite band are homozygous for the allele. For population abbreviations, see Table 1.

for the wild populations ranged from 0.660 to 0.682. This was a higher range than that of the estimated genotype data of hatchery populations, which ranged from 0.630 to 0.661. In total, 21 alleles were found to be unique to a single population (Table 2). Our unique allele analysis showed that hatchery populations had fewer unique alleles. However, despite these differences in genetic diversity, no statistically significant decrease in genetic variability was found in the hatchery strains compared with the wild population samples (Kruskal-Wallis test, $P > 0.05$).

Forty-eight cases of the observed genotype distribution were generally in accordance with the Hardy-Weinberg proportions; however, 12 (20%) remained significant after adjustment of P values using the sequential Bonferroni method ($P < 0.005$), and these disequilibriums (DHWE) were due to an excess of homozygotes (Table 2). Significant deviations were neither evenly distributed among samples or loci, nor were they associated with a particular locus or sample. Five deviations from equilibrium were observed at three loci in the wild populations, but seven cases were found at four loci in hatchery strains.

Genetic differentiation among populations

Small, but statistically significant, genetic differentiation (overall $F_{ST} = 0.0237$, $P < 0.05$) was observed among populations, indicating genetic heterogeneity among them. Table 3 presents the pattern of genetic differentiation among populations observed by comparing D_{CE} and mean pairwise F_{ST} values, based on the MS data. Significant genetic differentiation among populations was inferred between the wild and hatchery samples, but no significant differentiation was found among the wild populations. However, high heterogeneity and highly significant F_{ST} values (after Bonferroni correction) were obtained for all pairwise comparisons between hatchery and wild strains. Significant differentiation was also obtained for all pairwise comparisons of farmed population samples (Table 3). The NJ tree and dendrogram revealed that the wild populations formed one genetically distinguished cluster, but the hatchery strains did not (Figure 2). These findings were also evident in the PCA scatter plot (Figure 3). Together, the two axes explained 69% of total genetic variation. In this plot, wild population samples are in close proximity, whereas the other samples are more distant and in separate groups. Together, pairwise F_{ST} tests, NJ tree, and PCA of *S. inermis* samples revealed that Y, T, and N did not differ genetically from each other and were distinct from YH, TH, and NH, which differed genetically from each other.

Table 3. Chord distance (D_{CE}, below the diagonal) and mean F_{ST} estimates (above diagonal) among three wild and three cultured populations of *Sebastes inermis.*

Populations	Wild population			Hatchery strain		
	Y	T	N	YH	TH	NH
Y	-	0.0083^{NS}	0.0028^{NS}	0.0382***	0.0327***	0.0184*
T	0.0279	-	0.0079^{NS}	0.0334**	0.0253**	0.0178*
N	0.0212	0.0245	-	0.0295***	0.0182***	0.0160*
YH	0.0405	0.0496	0.0432	-	0.0517***	0.0438***
TH	0.0452	0.0446	0.0383	0.0478	-	0.0189*
NH	0.0406	0.0369	0.0312	0.0480	0.0314	-

D_{CE} (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 < 0.05; **P < 0.01; ***P < 0001; NS = not significant (P ≥ 005). For population abbreviations, see Table 1.

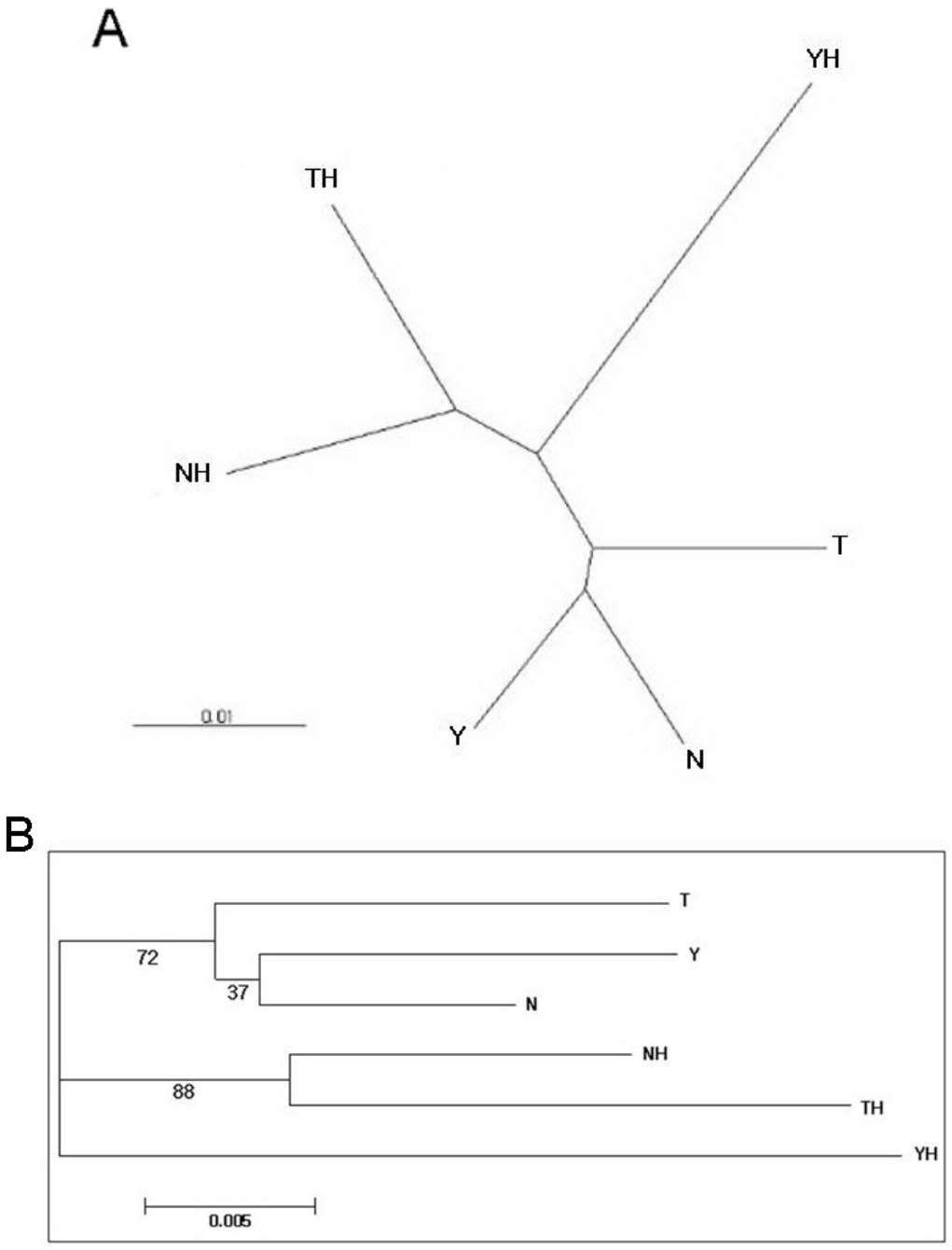

Figure 2. A. Neighbor-joining tree and **B.** dendrogram derived from chord distance (D_{CE}) matrix among three wild and three hatchery populations of *Sebastes inermis*. Bootstrap values are given as nodal values along the branches. For population abbreviations, see Table 1.

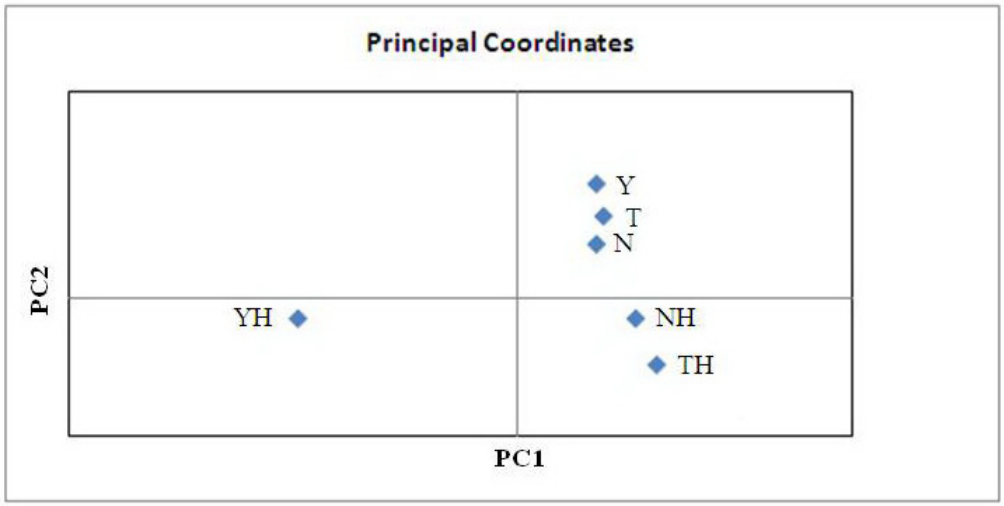

Figure 3. Principal component analysis, plotting the relationships of the studied *Sebastes inermis* wild and hatchery population samples, based on chord distances (D_{CE}) (Cavalli-Sforza and Edwards, 1967).

DISCUSSION

Although the loci and primers used in this study were originally described and designed for a congener (*S. schlegeli*), they all worked satisfactorily for *S. inermis*, as well as other Northeast Pacific species (An et al., 2009). This remarkable level of cross-species microsatellite conservation is a clear indication of the close molecular relationship of species *Sebastes*.

Molecular genetic diversity in fishes is associated with life history traits reflecting habitat types. In this study, the genetic variation of wild population samples of Korean *S. inermis* was low relative to that reported in other *Sebastes* species (Rocha-Olivares et al., 2003; Buonaccorsi et al., 2002, 2004), as well as other marine fishery species (DeWoody and Avise, 2000). However, similar genetic variability was reported in *S. maliger* (Wimberger et al., 1999) and *S. thompsoni* (Sekino et al., 2001).

Studies on the genetic variation of wild and hatchery samples are important, because common artificial production can lead to changes in allelic frequencies, which can have harmful consequences on the fitness-survival of wild populations. When the overall level of diversity of hatchery stocks was compared with that of the wild populations examined here, 94.1% of the allelic richness, on average, observed within the three wild samples was present in the hatchery samples, and the hatchery population displayed a slightly lower overall level of heterozygosity. Loss of genetic variation in hatchery strains, compared with wild populations, has been documented in several studies (Ryman and Ståhl, 1980; Liu et al., 2005; Shikano et al., 2008). The heterozygosity values of MS loci did not differ markedly between wild and hatchery populations, suggesting that allelic diversity is a more sensitive measure of genetic variation between wild and hatchery populations. Similar results have been reported previously (Norris et al., 1999; Karaiskou et al., 2009). However, no significant difference in the average N_A per locus or average H_E was observed. This indicates that these cultured black

rockfish stock have maintained considerable variation throughout the process of domestication. In aquaculture stocks, genetic variability is positively associated with the number of broodstocks used to produce stock. That rockfish are live-bearers has been combined with standard practices in Korean black rockfish hatcheries, which, in a spawning, typically use hundreds of potbellied females from cages culturing thousands of rockfish, to minimize the loss of genetic variability.

DHWE was observed in all population samples and at a number of loci. Population samples comprised same-year class individuals. Thus, this deviation could not result from temporal variability among different-year classes, and null or non-amplifying alleles may explain the heterozygote deficiencies in our data. In fact, MICRO-CHECKER analysis revealed the presence of null alleles at five loci, including four that DHWE detected. An admixture of more than two independent populations, nonrandom mating, or artificial selection forces during seed production and cultivation might account for the deviation from HWE in the hatchery population.

The genetic differentiation within and between the hatchery strains is probably due to genetic drift of breeding stocks after subsequent generations or the differing origins of fish used as broodstocks. Similar results have also been reported using both microsatellite and allozyme analyses of genetic differentiation observed between wild and proximal cultured populations (Hedgecock and Sly, 1990; Alarcón et al., 2004). However, some element of domestication or hatchery selection may have led to changes in the genetic composition of farmed strains compared with source populations.

Our study also analyzed the genetic relationship among the *S. inermis* samples. We found no differentiation among wild *S. inermis* populations, despite different sea basin origins. The lack of significant genetic differentiation between the wild populations we sampled is consistent with the lack of genetic divergence found in wild *S. inermis* from mtDNA sequence variability studies (Higuchi and Kato, 2002), as well as that of other *Sebastes* species (Rocha-Olivares et al., 2003; Gilbert-Horvath et al., 2006). This could result from sufficient gene flow during the black rockfish pelagic phase. Like that of many other benthic marine organisms, the life cycle of *S. inermis* includes a relatively long pelagic larval and early juvenile stage. The existence of separation-floating-type eggs is often assumed to facilitate large-scale larval dispersal, resulting in high levels of gene flow among populations, particularly when geographically close.

In conclusion, this study demonstrated that cross-species MS analysis can be used to compare the genetic variation of wild and hatchery populations of *S. inermis* and to estimate the genetic population structure of this species. Additional monitoring of genetic variability and continuous control of inbreeding in commercial breeding practices are required to promote the genetically sustainable management of the stock enhancement program. Such information could be used for the preservation and further genetic management of Korean black rockfish.

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