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Comparative genetic variability between broodstock and offspring populations of Korean starry flounder used for stock enhancement in a hatchery by using microsatellite DNA analyses

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ABSTRACT. Korean starry flounder, *Platichthys stellatus* (Pleuronectidae), is one of the most economically important fishery resources in Korea. We investigated the effect of current artificial reproduction in a hatchery facility, genetic divergence between the broodstock and their offspring populations of starry flounder in a hatchery strain to be stocked into natural sea areas was accessed using 9 polymorphic nuclear microsatellite DNA loci. High levels of polymorphism were observed between the 2 populations. A total of 96 alleles were detected at the loci, with some alleles being unique in the broodstock. Allelic variability ranged from 8 to 17 in the broodstock and from 7 to 12 in the offspring population. Average observed and expected heterozygosities were estimated at 0.565 and 0.741 in the broodstock samples and 0.629 and 0.698 in the offspring population, respectively. Although no statistically significant reductions were found

in heterozygosity or allelic diversity in the offspring population, a considerable loss of rare alleles was observed in the offspring population compared with that in the broodstock. Significant genetic difference was detected between the broodstock and offspring populations (F_{ST} = 0.021, P < 0.05). These results suggest that more intensive breeding practices for stock enhancement might have resulted in a further decrease of genetic diversity. Thus, genetic variations of broodstock and progeny should ideally be monitored in both breeding and release programs as a routine hatchery operation in order to improve the starry flounder hatchery management. This information might be useful for fishery management and aquaculture industry of *P. stellatus*.

Key words: Korean starry flounder; *Platichthys stellatus*; Broodstock; Artificial reproduction; Microsatellite loci; Stock enhancement

INTRODUCTION

Korean starry flounder, *Platichthys stellatus* (Pleuronectiformes: Pleuronectidae), is a cold water marine fish found in the North Pacific, spanning Korea, Japan, Sea of Okhotsk, Bering Sea, and Alaska to California (Kang et al., 2012). In Korea, *P. stellatus* is an important fishery resource for prospective aquaculture diversification. However, the annual catch of this fish has fluctuated and continuously declined. Increasing the harvest yield requires that efforts should be focused on aquaculture production and stock enhancement. Recently, the aquaculture industry of starry flounder farming is booming in areas surrounding the East Sea of Korea because of the establishment of a mass production technique for seedlings. Due to the steady production decrease, a stock enhancement program that involves the release of hatchery-reared juveniles to the natural environment was started by the Korean government in the early 2000 and is still ongoing (KFRA, 2010; NFRDI, 2010).

Starry flounder farming is a key part of aquaculture in eastern Korea. Seeds of starry flounder in this region are produced exclusively in hatcheries. Typically, almost all starry flounder hatcheries maintain hundreds of males and females from both wild-caught and captive-cultured sources of *P. stellatus* as the broodstock for artificial reproduction for stock enhancement. However, breeding has persisted for multiple generations with little introduction of exotic broodstocks, due to the difficulty in collecting large numbers of sexually mature wild *P. stellatus*. Furthermore, in practice, offspring starry flounders usually breed without any consideration to the mating system. Thus, there were concerns that genetic variability in hatchery stocks might have been lost during artificial propagation. Loss of genetic variation leads to potential harmful effects on various commercially important traits such as survival and growth, which can damage the aquaculture process (Allendorf and Pyman, 1987). Intensive stocking practices with inappropriate hatchery procedures might result in detrimental genetic impacts on the wild stock accompanied by risk to the continued exploitation of the resource. 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 a hatchery (Kohlmann et al., 2005; An et al., 2011b, 2013; Wang et al., 2011). Genetic diversity of wild and cultivated *P. stellatus* stocks was

recently reported (An et al., 2011a). Research data on the standard breeding practices in a hatchery are needed for maintaining cultivated stocks for the conservation of diversity and minimization of inbreeding for future aquaculture development. However, little effort has been undertaken to understand genetic divergence between the broodstock and their offspring populations of Korean starry flounder produced in a hatchery facility, despite the concerted efforts aimed at artificial propagation. Therefore, genetic diversity should ideally be monitored during both breeding and release programs as a routine hatchery operation in order to improve starry flounder hatchery management.

Molecular markers have proved to be an exceptional indicator of genetic variation within and between populations of many fishery animals, including fish (Yoon et al., 2011; Han et al., 2012; Hong et al., 2012; Lee and Hur, 2012). Especially, microsatellite (MS) DNA markers or short tandem repeats (STRs) are well-known hyper-variable genetic markers that have remarkable discrimination power for the evaluation of genetic diversity across various marine species (Hauser and Carvalho, 2008; An et al., 2011c, 2012; Blanco Gonzalez et al., 2012).

The present study aimed to characterize genetically the broodstock and offspring of a local government hatchery at Uljin, Korea, that releases the produced juveniles to the wild as a tool for stock enhancement, in order to determine whether current management strategy of hatchery stocks in a hatchery facility lead to reduced genetic variation of *P. stellatus.*

MATERIAL AND METHODS

Sample collection and DNA extraction

Samples of fin-clip tissue (approximately 1 cm^3) were obtained from a local government hatchery at Uljin, Korea, in which the offspring of starry flounder to be released at several sites along the eastern coast are produced every year. At this facility, approximately 300 breeders between the ages of 2 and 5 years are maintained for reproduction. In 2008, 82 broodstock starry flounders were selected for reproduction according to sexual maturity and health condition. All these fishes were sampled. After the mating event, their offspring starry flounders were reared in a tank. Immediately before stocking, 50 offspring starry flounders were randomly sampled. In general, both wild-caught and captive-cultured sources of the broodstock were used for artificial reproduction. However, one hatchery manager informed that breeding has persisted for multiple generations with very little introduction of exotic broodstocks, due to the difficulty in collecting large numbers of sexually mature wild *P. stellatus*. No details regarding the founding and maintenance of the farmed strain are available; however, their original parents were held at a farm on the eastern coast of Korea. All samples were stored in 2 mL 99% ethanol at 4°C until DNA extraction. For genotyping, total DNA from fin-clips of each sample was extracted using a MagExtractor-Genomic DNA Purification Kit (TOYOBO, Osaka, Japan) by using an automated DNA extraction system, MagExtractor MFX-2100 (TOYOBO). The genomic DNA extracted was stored at -20°C until genotyping.

Microsatellite genotyping

Nine highly variable microsatellite loci previously characterized (KPs1, KPs2, KPs15,

KPs17A, KPs20, KPs25, KPs32, KPs33, and KPs36; An et al., 2011a) were selected for the present study (Table 1). A total of 132 starry flounders from the 2 populations were typed. Primer sequences, microsatellite repeat sequence, and the optimal annealing temperature for each locus are listed in Table 1. The forward primer from each primer set was 5'-fluorescent labeled with 1 of 3 dyes: 6-FAM, HEX, or NED (PE Applied Biosystems, Foster City, CA, USA). PCR amplification of 9 microsatellite loci was carried out using an RTC 200 instrument (MJ Research, Watertown, MA, USA) in 10 µL solution containing 1050 ng DNA, 1X ExTaq buffer, 0.2 mM dNTPs, 10 pmol each primer, and 0.25 U Taq DNA polymerase (Takara, Shiga, Japan). The amplification protocol included an initial denaturation for 11 min at 95°C, followed by 35 cycles of 1 min at 94°C, 1 min at the optimal annealing template listed in An et al. (2011a), and 1 min at 72°C, with a final extension step of 5 min at 72°C. For genotyping, 1 µL PCR product was added to each reaction containing formamide with a size standard GeneScan-400HD [ROX] (Applied Biosystems) and electrophoresed using an ABI3130 DNA sequencer (Applied Biosystems). The fragment length of the PCR products was determined using the GeneMapper software (Version 4.0, Applied Biosystems).

Table 1. Nine microsatellite loci sequences of starry flounder (*Platichthys stellatus*) used in this study, core repeats and their specific annealing temperatures used in PCR amplification.

Locus	Repeat motif	Primer sequence (5'→3')	Ta (°C)	GenBank accession No.
KPs1	$(CA)_{11}$	CAGCAGTAAGAGTGTGTCCTG hex TTCAGCCTGTTTTCTGTCAT	55	EF157643
KPs2	$(TG)_{12}$	TTAGGGGTGGGACAGACT hex GTCATCAGATGGGAGAAAGAT	55	EF157644
KPs15	$(TG)_5TA(TG)_8TC(TG)_4$	GAGCCAGACCTCTCATGTTAC fam CGCTTCCATGTGAACCAG	60	EF157648
KPs17A	$(TG)_5TC(TG)_9$	CAACCACGTTATCCTCTGTG fam CCAGAATAAATCTCATGCTCA	60	EF157649
KPs20	$(CA)_{14}$	TGGGCAACTACGTACACACTA fam GCCGACATTACAAAAACAAA	58	EF157651
KPs25	$(TG)_6TA(TG)_8TA(TG)_{10}$	TGTTATCGGGTGTTGATTGT aned GTTGATTGTGAAACGCTGTT	60	EF157653
KPs32	$(TG)_8TT(TG)_7TA(TG)_{22}$	TTAAATAAGTGTCTGGGGATT hex GCCACACTTCTGCTTCTG	55	EF157657
KPs33	$(CA)_9$-$(CA)_{11}$	TTTCACTTCTCTTTGGGTTAC ned GGCAGACTGATTCCTCATT	60	EF157658
KPs36	$(TG)_4TT(TG)_{13}$	ATGTGCCCAATAAAACAAAC hex CTAAGCCCTAGAGCAAACAGT	58	EF157659

Ta = optimal annealing temperature.

Statistical analyses

The possible presence of null alleles and genotyping errors caused by stuttering and/or large-allele dropout were tested using MICRO-CHECKER (ver. 2.2.3; 1000 randomizations; van Oosterhout et al., 2004). To analyze the variation in microsatellite loci in the samples, the number of alleles per locus (N_A), allelic richness (A_R), allele size range (S), and number of unique alleles (U) were determined for each population at each locus by using the program FSTAT version 2.9.3.2 (Goudet, 1995). The A_R measures the number of alleles independent of the sample size. The genetic heterozygosity between the population samples was estimated by calculating unbiased expected and observed heterozygosity values (H_E and H_O, respectively; Nei, 1978) by using the ARLEQUIN 3.0 (Excoffier et al., 2005) software. Differences in genetic diversity parameters were tested

using nonparametric analysis (Wilcoxon signed-rank test; Wilcoxon, 1945). Deviations from Hardy-Weinberg equilibrium (HWE) at each locus were tested using a test analogous to the Fisher exact test by using the Markov-chain procedure of ARLEQUIN (the Markov-chain parameters used were: steps, 100,000; dememorization, 10,000). ARLEQUIN was also used to assess linkage disequilibrium for all pairs of loci, whose empirical distribution is obtained by a permutation procedure (Slatkin and Excoffier, 1996). An inbreeding coefficient (Weir and Cockerham, 1984) was also estimated to measure the HWE departures evaluating the probabilities through random permutation procedures (minimum 10,000 permutations). Significance levels associated with the HWE analysis was adjusted for multiple tests by using the sequential Bonferroni correction technique (Rice, 1989). The genetic distance between populations was estimated on the basis of the chord distance, D_{CE} (Cavalli-Sforza and Edwards, 1967).

The extent of population subdivision was investigated by calculating fixation indices. Differentiation between populations was characterized using F_{ST} (Weir and Cockerham, 1984) and R_{ST} (Slatkin, 1995; Michalakis and Excoffier, 1996) estimates calculated using the computer program GENEPOP ver. 4.0 (http://kimura.univ-montp2.fr/~rousset/Genepop.htm). R_{ST} incorporates the correlation of the weighted mean allele size expressed as the number of tandem repeats. The sequential Bonferroni correction was applied to derive significance levels for the analysis involving multiple comparisons (Rice, 1989).

RESULTS

Genetic diversity of the broodstock and their offspring samples

Samples of 82 broodstock and 50 offspring *P. stellatus* collected from a hatchery, around the eastern coast of Korea, were screened for variation at the 9 polymorphic microsatellite loci. The 9 primer sets were polymorphic across the 2 Korean starry flounder populations studied, and the levels of polymorphisms (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, and these replications produced identical results in each trial.

The MICRO-CHECKER analysis showed that most loci might have been influenced by one or more null alleles in both samples. Loci KPs1 and KPs20 appeared to be influenced in both the broodstock and their offspring samples, indicating that using loci KPs1 and KPs20 for population genetic analyses that assume HWE might prove to be problematic. Thus, a global multilocus F_{ST} value was estimated with and without these loci. However, factors existed that indicated that the other loci were affected by null alleles in only 1 sample. These loci were used in this study because there was no indication of an allele scoring error caused by stuttering and large-allele dropout. However, the presence of null alleles could be problematic for the estimates of Nei's genetic distance (Nei, 1978); thus, Cavalli-Sforza distance was used because this distance is less affected by null alleles (Chapuis and Estoup, 2006).

Genetic variability in each population is shown in Table 2. A total of 96 different alleles were observed over all 9 loci in the samples; the number of alleles varied from 4 at loci KPs20 to 17 at locus KPs25 and KPs32 (Table 2). Fewer alleles were found in the offspring population compared with those in the broodstock sample (Wilcoxon signed-rank test, P >

0.05). The overall allelic richness varied from 5.97 to 13.53 (Table 2), with the broodstock population showing higher allelic richness than the offspring population (Wilcoxon signed-rank test, P > 0.05). In total, 22 alleles were found to be unique to the broodstock population (Tables 2 and 3). Because the number of individuals analyzed differed between the populations, these results should be interpreted with caution. Despite these differences in genetic diversity, however, no clear difference in the average measures was observed between broodstock and their offspring samples (Wilcoxon signed-rank test, P > 0.05).

Table 2. Allelic variability observed at nine microsatellite loci in broodstock and their offspring *Platichthys stellatus* populations.

Population (No.)		Microsatellite loci									
		KPs1	KPs2	KPs15	KPs17A	KPs20	KPs25	KPs32	KPs33	KPs36	Mean
	F_{ST}	0.014	0.039	0.005	0.004	0.07	0.023	0.018	0.018	0.008	0.021
Broodstock (82)	N_A	11	8	8	8	9	17	17	8	10	10.67
	A_R	9.62	7.01	7.68	7.94	7.95	15.76	15.05	7.99	9.76	9.86
	S	140-196	138-166	118-152	68-84	118-134	204-332	152-206	274-294	182-216	
	R	56	28	34	16	16	128	54	20	34	
	U	4	1	1	0	5	6	5	0	2	2.67
	H_E	0.806	0.576	0.712	0.751	0.602	0.868	0.857	0.74	0.756	0.741
	H_O	0.610	0.634	0.793	0.232	0.329	0.646	0.707	0.512	0.622	0.565
	F_{IS}	0.245*	-0.103	-0.114	0.693*	0.455*	0.256*	0.176	0.309*	0.178	0.034
Offspring (50)	N_A	7	7	7	8	4	11	12	8	8	8
	A_R	7	7	7	8	4	11	12	8	8	8
	S	140-170	140-166	118-146	68-84	120-130	266-332	154-206	274-294	182-216	
	R	30	26	28	16	10	66	52	20	34	
	U	0	0	0	0	0	0	0	0	0	0
	H_E	0.770	0.682	0.727	0.688	0.417	0.798	0.876	0.633	0.695	0.698
	H_O	0.580	0.680	0.820	0.620	0.200	0.720	0.920	0.520	0.600	0.629
	F_{IS}	0.249*	0.002	-0.129	0.100	0.523*	0.099	-0.051	0.18	0.137	0.048
Mean all population	N_A	9	7.5	7.5	8	6.5	14	14.5	8	9	9.33
	A_R	8.31	7	7.34	7.97	5.97	13.38	13.53	7.99	8.88	8.93
	H_E	0.788	0.629	0.720	0.720	0.510	0.833	0.867	0.687	0.726	0.72
	H_O	0.595	0.657	0.807	0.426	0.265	0.683	0.814	0.516	0.611	0.597

No. of alleles per locus (N_A), allellic richness (A_R), size in bp of alleles (S), allelic size range (R), No. of unique alleles (U), expected heterozygosity (H_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}) are given for each population and locus. Calculations assume that individuals with one microsatellite band are homozygous for the allele. *Significant deviation from Hardy-Weinberg equibrium after Bonferroni's corrections (P, initial $\alpha = 0.05/9 = 0.006$).

The H_O ranged from 0.265 at locus KPs20 to 0.814 at KPs32, whereas the H_E varied from 0.510 to 0.867 at the same loci (Table 2). In terms of heterozygosity, no significant difference was detected between the broodstock and offspring samples. Inbreeding coefficients (F_{IS}) varied among markers from -0.129 (KPs15) to 0.523 (KPs20) in the offspring samples, and from -0.114 (KPs15) to 0.693 (KPs17A) in the broodstock samples. Average F_{IS}, including all markers, was 0.048 in the offspring sample and 0.034 in the broodstock sample.

Significant departures from HWE after Bonferroni correction (P < 0.006) were found at 2 loci (KPs1 and KPs20) in the offspring samples, and at 5 loci (KPs1, KPs17A, KPs20, KPs25, and KPs33) in the broodstock samples, indicating that deviations from HWE were due to heterozygote deficiency.

The allele frequencies of all 9 microsatellites in each sample are shown in Table 3 and Figure 1. The allele frequency distributions indicated the presence of 28 rare alleles (frequency < 5%) among a total of 72 alleles over all loci (mean = 38.9%) in the offspring

sample, whereas 53 rare alleles among a total of 96 alleles (mean = 55.2%) were observed in the broodstock sample. Rare alleles were detected at most loci and were not associated with a particular locus in either population. There was no significant linkage disequilibrium between loci pairs ($P > 0.05$).

Table 3. Frequency of each microsatellite allele in broodstock and offspring *Platichthys stellatus* samples in a hatchery.

Loci	Allele	Broodstock	Offspring	Loci	Allele	Broodstock	Offspring
KPs1	140	0.018	0.010	KPs25	204	0.006	0.000
	142	0.305	0.370		266	0.024	0.020
	144	0.006	0.000		280	0.043	0.000
	148	0.006	0.070		282	0.091	0.070
	150	0.043	0.100		284	0.012	0.000
	158	0.171	0.060		286	0.018	0.070
	164	0.232	0.250		290	0.030	0.020
	168	0.012	0.000		294	0.091	0.280
	170	0.091	0.140		298	0.104	0.040
	174	0.110	0.000		308	0.030	0.030
	196	0.006	0.000		310	0.018	0.030
					314	0.067	0.000
KPs2	138	0.018	0.000		316	0.006	0.000
	140	0.006	0.010		322	0.299	0.330
	144	0.140	0.090		324	0.012	0.000
	146	0.616	0.450		330	0.061	0.090
	148	0.165	0.330		332	0.085	0.020
	150	0.037	0.060				
	152	0.006	0.020	KPs32	152	0.006	0.000
	166	0.012	0.040		154	0.030	0.050
					156	0.098	0.100
KPs15	118	0.250	0.180		158	0.030	0.000
	120	0.018	0.010		172	0.006	0.000
	124	0.018	0.010		174	0.006	0.000
	126	0.445	0.420		176	0.165	0.120
	128	0.152	0.250		178	0.274	0.130
	132	0.085	0.070		180	0.055	0.090
	146	0.018	0.060		182	0.012	0.010
	152	0.012	0.000		194	0.159	0.210
					196	0.012	0.050
KPs17A	68	0.018	0.030		198	0.030	0.000
	70	0.201	0.110		200	0.037	0.040
	72	0.061	0.010		202	0.018	0.010
	74	0.037	0.030		204	0.055	0.180
	76	0.055	0.020		206	0.006	0.010
	78	0.427	0.510				
	82	0.134	0.180	KPs33	274	0.079	0.070
	84	0.067	0.110		280	0.451	0.560
					282	0.061	0.010
KPs20	118	0.012	0.000		284	0.037	0.020
	120	0.024	0.060		286	0.128	0.220
	122	0.024	0.010		288	0.177	0.050
	124	0.006	0.000		292	0.037	0.060
	126	0.006	0.000		294	0.030	0.010
	128	0.506	0.740				
	130	0.378	0.190	KPs36	182	0.445	0.510
	132	0.024	0.000		184	0.073	0.030
	134	0.018	0.000		188	0.152	0.140
					190	0.098	0.010
					192	0.079	0.050
					194	0.012	0.000
					196	0.067	0.120
					198	0.018	0.000
					214	0.030	0.120
					216	0.024	0.020

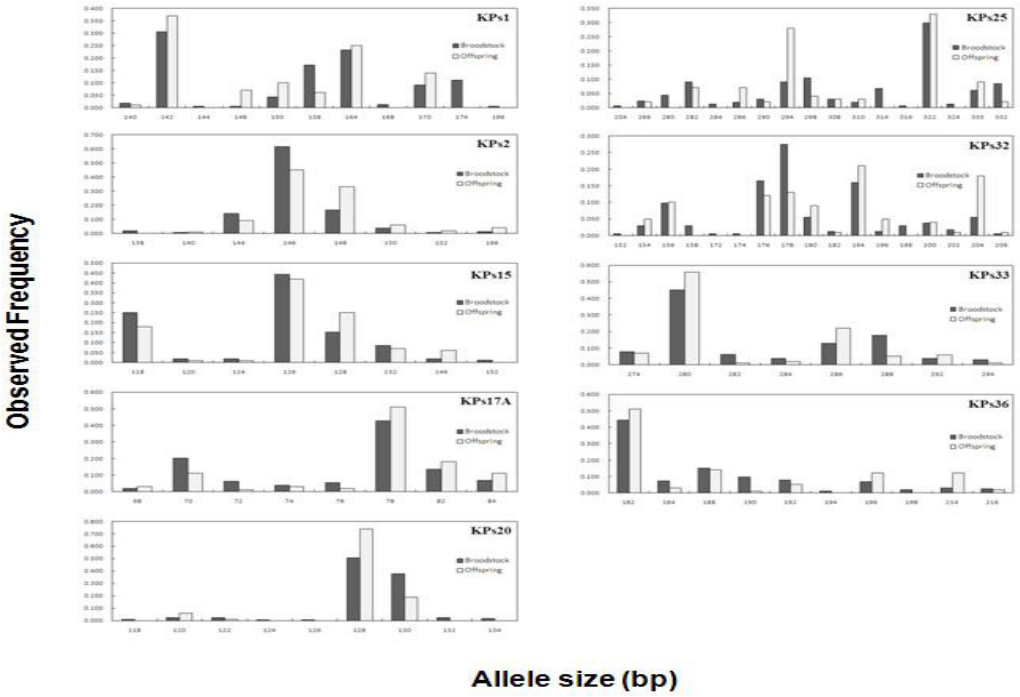

Figure 1. Allele size frequency distributions of the nine microsatellite loci of *Platichthys stellatus* used in this study.

Genetic variation between samples

Genetic differentiation between broodstock and offspring populations in a hatchery was estimated using F_{ST} and R_{ST} estimates. The global multilocus F_{ST}, including all loci, was estimated to be 0.021 ($P < 0.01$), and the R_{ST} value was 0.026 ($P < 0.01$). F_{ST} estimates were significantly different between broodstock and offspring populations irrespective of whether the 2 loci with potential null alleles (KPs1 and KPs20) were included. When 2 loci were excluded, the global multilocus F_{ST} was estimated to be 0.016 ($P < 0.01$), and the R_{ST} value was 0.027 ($P < 0.01$). The significant F_{ST} and R_{ST} estimates indicate genetic differentiation between these broodstock and their offspring starry flounder samples.

DISCUSSION

In this study, the genetic variation of hatchery stock samples of *P. stellatus* (mean N_A = 9.33 and mean H_E = 0.72) was lower than the reported ones (mean N_A = 19.96 ± 6.6, mean H_E = 0.77 ± 0.19 averaged from 12 species) for other marine fish species (DeWoody and Avise, 2000). Similar genetic variability has been reported for these species as well as for another marine species, the Korean black rockfish, which might indicate that these demersal fish are less diverse compared to other migratory fishes (An et al., 2011a,c).

When the level of diversity in offspring population was compared with that in the broodstock samples, no significant difference in the average N_A per locus or average H_E was

observed. However, on average, 81.1% of the allelic richness observed within the broodstock samples was present in the offspring samples, and the offspring population showed a slightly lower level of heterozygosity. Reduction of genetic variability in hatchery populations compared to a broodstock or a natural population has been reported in other cultivated fish species (Porta et al., 2006). The results of this study were consistent with these findings. In these reports, the cause of reduction of genetic variability was inferred to be the actual number of parents contributing to the offspring being less than that of the total broodstock or natural population available. Tessier et al. (1997) reported an extreme case in which stocked offspring caused major genetic drift and a 50% reduction in effective population size of a wild Atlantic salmon population. No detailed information was available regarding the number of parents that actually contributed to the production of the offspring used in this study. Thus, the results obtained should be evaluated with caution.

Significant deviations from HWE in the direction of deficiencies of heterozygotes were detected in both the broodstock and their offspring samples, even after sequential Bonferroni's correction for multiple tests. A similar phenomenon was also observed in a previous microsatellite analysis (Ortega-Villaizán Romo et al., 2005). Null or non-amplifying alleles could possibly account for the heterozygote deficiencies in the data. Indeed, our MICRO-CHECKER analysis revealed the presence of null alleles at most loci, with a significant heterozygote deficit. The presence of null alleles has also been reported for numerous other fishery animals (Frankham et al., 2002; Keller and Waller, 2002). However, admixture of more than 2 independent populations, nonrandom mating, or artificial selection forces during seed production and cultivation might account for the deviation from HWE in both the broodstock and their offspring populations.

There was significant genetic differentiation detected by F_{ST} and R_{ST} values between the broodstock and offspring starry flounder samples. This difference is likely to be a result of the reduction in the effective number of contributing parents. Considering that this study was limited by the number of populations screened, the genetic diversity parameters for each population might be explained by the data from additional populations. Therefore, our results should be interpreted with caution. Further study is required to assess the influence of the standard breeding practices in a hatchery of this important fishery species.

The preservation of genetic variation is an important consideration in maintaining any hatchery stock to provide fish to maintain wild populations. Continued intensive breeding practices for stock enhancement might lead to decreased genetic variability if the number of effective breeders is not maintained. Maintenance of levels of genetic variation within hatchery stocks comparable to wild source populations requires good broodstock management practices. In hatchery strains, use of unrelated fish from the wild as broodstock is generally recommended because they can produce offspring that have a higher fitness in the wild than offspring from older, domesticated hatchery stocks, even though first-generation hatchery fish still have reduced fitness in the wild (Christie et al., 2012). However, this is often difficult to achieve due to economic constraints. Instead, a subset of hatchery-based fish is normally used as broodstock. Historically, hatchery managers preferred the use of fish born in hatcheries as broodstock to create future generations, because whatever trait they had that allowed them to succeed in the hatchery helped produce thousands of apparently healthy young offspring. However, this practice can have unintentional negative effects on the genetic variability of the broodstock (Allendorf and Pyman, 1987). Parental similarity is negatively related to adult re-

productive success, and that, wherever sample sizes are large, the relationships become significantly negative (Amos et al., 2001). Thus, periodically introducing new genetic material from local wild stocks is necessary. This infusion would preserve the starry flounder gene pool and would enable hatchery production of fish that could perform well when returned to the wild. In addition, even if founder specimens of broodstock are genetically intact, lack of a proper management strategy can lead to inbreeding and rapid decline in diversity at the gene level. Hence, continued genetic monitoring of bloodstock is warranted in artificial seed production.

A desirable characteristic of a stock enhancement program is to release genetically similar fish to a wild population or a broodstock containing unrelated fish from the wild. For Korean starry flounder, many rare alleles at 9 microsatellite DNA loci were found in the broodstock and some were not observed in the progeny produced for release. Probability of loss of rare alleles is very high (Allendorf, 1986), and all alleles lost in the offspring were rare alleles. In fact, the loss of alleles is more important than the change in allele frequencies, because the latter can be changed again by random drift, but a lost allele cannot be recovered. For the proper management of stock enhancement programs, production of progeny should be based on well-organized broodstock management strategies. Genetic factors are of vital importance in the production of good quality seed of marine animals for stocking in the sea. Genetic integrity of wild populations should be protected from the impact of hatchery releases through a carefully planned broodstock management strategy. Unknown and known genetic changes and possible loss of genetic variation in broodstock and progeny should be monitored. Molecular tools such as nuclear DNA markers are a good monitoring tool for broodstock management. This information will be useful to evaluate the feasibility of the enhancement program to maintain the genetic diversity of the wild population as well as to improve the management of the hatchery for the following season.

In summary, genetic diversity analysis revealed changes in the genetic composition between the broodstock and offspring starry flounder populations in a hatchery in Korea. The offspring starry flounder produced for release in a stock enhancement program showed some level of genetic differentiation, compared with the broodstock samples. Thus, for an adequate stocking strategy of starry flounder, periodic genetic evaluations of bloodstock, progeny, and target populations are necessary. Such genetic monitoring can be useful for the preservation and further genetic management of the Korean starry flounder.

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