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New polymorphic microsatellite markers in the Korean mi-iuy croaker, *Miichthys miiuy*, and their application to the genetic characterization of wild and farmed populations

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Eighteen new polymorphic microsatellite markers were developed for the Korean mi-iuy croaker (*Miichthys miiuy*, Perciformes, Sciaenidae), and allelic variability was compared between a wild population in Mokpo, Korea, and a hatchery population in Tongyeong, Korea. All loci were amplified readily and demonstrated allelic variability, with the number of alleles ranging from 5 to 37 in the wild population, and from 4 to 12 in the farmed population. The average observed and expected heterozygosities were estimated, respectively, to be 0.74 and 0.78 in the hatchery population samples, and 0.79 and 0.86 in the wild samples. These results indicate lower genetic variability in the hatchery population compared with the wild population, and significant genetic differentiation between the wild population and the hatchery samples ($F_{ST} = 0.058$, $P < 0.001$). These microsatellite loci may be valuable for future population genetic studies, monitoring changes in the genetic variation within stocks in a commercial breeding program, conservation genetics, and molecular assisted selective breeding of the mi-iuy croaker in the future.

Keywords: Korean mi-iuy croaker; *Miichthys miiuy*; microsatellite loci; genetic marker; genetic variability

Introduction

The mi-iuy croaker, *Miichthys miiuy* (Perciformes Sciaenidae), is found in countries surrounding the Northeast Pacific Ocean, including the western coast of Korea, the western Japan Sea, and the East China Sea. Typically, it inhabits coastal waters with mud to sandy mud bottoms (Chan et al. 1974). In Korea, *M. miiuy* is an important fishery resource and it has been regarded as a target marine fish species for prospective aquaculture diversification. However, the production of this important fishery species has declined year after year due to over-fishing, pollution, coastal construction, and other factors. To increase the harvest yield, efforts have been focused on resource enhancement. Complete culturing, including reproduction control, captive spawning, hatching, and larval and juvenile rearing became possible in 2006, and, recently, artificially hatched juveniles of *M. miiuy* were released into Korean west coastal breeding grounds.

The mass release of *M. miiuy* juveniles reared in hatcheries is expected to have an immediate effect on stock abundance, but it could also cause changes in the genetic structure of wild populations. Reduced genetic diversity has been related to a loss of adaptation to new environments (Allendorf and Ryman 1987); thus, monitoring the genetic differences between hatchery stocks and wild populations is recommended to preserve genetic variation in natural populations (FAO 1993). Furthermore, understanding the patterns of genetic variation is becoming more important for conservation, management, and remediation efforts for marine communities generally.

Statistical methods for the analysis of population genetics can be used to determine the breeding success of the mi-iuy croaker for sustainable fishery management purposes. These tests, however, can often only be discerned using highly variable molecular markers, such as microsatellites. Microsatellite (MS) DNA markers or short tandom repeats (STRs) represent a suitable tool for monitoring the changes in the genetic variation of farmed stocks, parentage assignment, and evaluation of genetic diversity and structure of various marine species for the improvement of fisheries and resource conservation because of their high degree of variability (Desvignes et al. 2001; Fritzner et al. 2001; An et al. 2008; Ahn et al. 2011). Despite the strong commercial interest in the mi-iuy croaker in Korea, to date no reported study has focused on the genetic variability or population structure of this species. Recently, 12 microsatellite markers for the mi-iuy croaker were characterized (Wang et al. 2010), but they showed relatively small alleles (six loci have less than five alleles) and linkage disequilibrium at six loci. Therefore, a large number must be developed and screened to identify a suit of loci that are powerful and efficient for population genetic analyses.

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In the present study, we developed 18 polymorphic microsatellite primer sets from an enriched *M. miiuy* DNA library, and examined the genetic variability at these loci in a wild population and a hatchery population of this species.

Materials and methods

Library construction and sequencing

The TNES-urea buffer method (Asahida et al. 1996) was used to isolate high-molecular-weight DNA from fin tissue samples of an individual mi-iuy croaker sampled from Mokpo, Korea.

A partial genomic library enriched for CA repeats was constructed using a slightly modified enrichment procedure with pre-hybridization PCR amplification, as described previously (Hamilton et al. 1999). Extracted DNA (20 μg) was digested with the restriction enzymes *Alu*I, *Rsa*I, *Nhe*I, and *Hha*I (New England Biolabs, Beverly, MA, USA). DNA fragments in the range 300–800 bp were isolated and purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The selected fragments were ligated to an adaptor (SNX/SNX rev linker sequences). Linker-ligated DNA was amplified using SNX as a linker-specific primer for PCR. For enrichment, the DNA was denatured and biotin-labeled repeat sequences, $(CA)_{12}$GCTTGA (Li et al. 2002), were hybridized to the PCR products. The hybridization complex was then lifted out using streptavidin-coated magnetic spheres (Promega, Madison, WI, USA). After washing, the bound enriched DNA was eluted from the magnetic spheres and re-amplified with an adaptor sequence primer. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen).

Purified PCR products were digested with *Nhe*I, cloned using *Xba*I-digested pUC18 vector (Pharmacia, Piscataway, NJ, USA), and transformed into *Escherichia coli* DH5α competent cells. White colonies were screened for the presence of a repeat insert using PCR with universal M13 primers and non-biotin-labeled dinucleotide primers. PCR products were examined on 2% agarose gels and inserts producing two or more bands were considered to contain a microsatellite locus. Positive clones were cultured and purified. Plasmids from insert-containing colonies were recovered using the QIAprep Spin Miniprep Kit (Qiagen) and sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (ver. 3.1; Applied Biosystems, Foster City, CA, USA) and an automated sequencer (ABI Prism 310 Genetic Analyzer; Applied Biosystems).

Primer design and genotyping

Primers were designed based on sequences flanking the microsatellite motifs using the OLIGO software package (ver. 5.0; National Biosciences Inc., Plymouth, MN, USA). Newly designed PCR primer pairs were tested to optimize annealing temperatures; a gradient PCR with a 50–60°C range was performed on a set of samples from eight individual mi-iuy croakers captured at Mokpo, Korea. PCR amplification was performed in a 10-μL reaction volume containing 0.25 U of *Extaq* DNA polymerase (TaKaRa Biomedical Inc., Shiga, Japan), 1 × PCR buffer, 0.2 mM dNTP mix, 10 pmol of each primer (the forward primer of each pair was 5′-end-labeled with 6-FAM, NED, and HEX dyes; Applied Biosystems), and 100 ng of template DNA, using a PTC 200 DNA Engine (MJ Research, Waltham, MA, USA). PCR conditions were as follows: 11 min at 95°C, followed by 35 cycles of 1 min at 94°C, 1 min at the annealing temperature (listed in Table 1), and 1 min at 72°C, with a final extension of 5 min at 72°C. Microsatellite polymorphisms were screened using an ABI PRISM 3100 Automated DNA Sequencer (Applied Biosystems), and alleles were designated according to PCR product size, relative to a molecular size marker (GENESCAN 400 HD [ROX]; Applied Biosystems). Fluorescent DNA fragments were analyzed using the GENESCAN (ver. 3.7) and GENOTYPER (ver. 3.7) software (Applied Biosystems).

Sample comparisons

Samples of 53 wild mi-iuy croakers were collected from Mokpo, Korea, between January and March 2008, and 51 farmed samples were obtained in July 2007 from a hatchery-reared population in Tongyeong, Korea, which was used as a broodstock for artificial reproduction. Although the hatchery population had been reared continuously, details of their origins and records were unavailable. Total DNA for genotyping was extracted using a MagExtractor-genomic DNA Purification Kit (TOYOBO, Osaka, Japan), according to the manufacturer's protocol for MagExtractor MFX–2100 (TOYOBO). Extracted genomic DNA was stored at −20°C until use.

Samples were screened for variation at the newly developed microsatellite loci. MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004) was used to detect genotyping errors due to null alleles, stuttering, or allele dropout (1000 randomizations). As genetic diversity statistics, the number of alleles per locus (N_A), size in bp of alleles (S), frequency of the most common allele (F), and number of unique alleles (U) were determined for each local sample at each locus, using the program GENEPOP (ver. 4.0; http://kimura.univ-montp2.fr/

Table 1. Characteristics of the 22 microsatellite loci isolated from *Miichthys miiuy*.

Locus	Repeat motif	Primer sequence (5′→3′)	*T*a (°C)	*Genebank accession no.*
KMn183	$(CA)_{12}$	F: GCCCTGGTATTCATTCTCT **6-fam** R: ACGGCTCTGTTGTAAAACTTA	*61*	FJ210960
KMm22	$(CA)_5GA(CA)_{14}$	F: TAACTGATGCACAGGTGATG **ned** R: CACATTGATCCACAACAGAAT	*61*	FJ210961
KMm216	$(GT)_{12}$	F: GAAGCACCAGGTAAGAGACA **6-fam** R: AGCAGTGAACTGTTTGAATGA	*65*	FJ210962
KMm218	$(GT)_{11}$	F: AACATTTCGCTCCAGATAAT **ned** R: AGTCAGCGATCAATAAAACAA	*61*	FJ210963
KMm236	$(CA)_5$-$(GT)_{15}$-$(GT)_6$	F: GTAAATGCGTCAGTCTGGAAG **6-fam** R: AAACATAGTCCCCGTGTCTT	*63*	FJ210964
KMm241	$(GT)_{13}$	F: ACGTCGTGTTATGTTCTGTTG **6-fam** R: AAAGTCCCCTGTTCACCAT	*61*	FJ210965
KMm244	$(GT)_{14}$	F: GGTTATATGATCCACGGTGAT **hex** R: GCTGACCACAGTTTCCTCTC	*65*	FJ210966
KMm270	$(CA)_{12}$	F: AATACGACGAAAATCAATGTT **hex** R: GGGCAGTTTTAAATC TCTGTA	*63*	FJ210967
KMm274	$(CA)_6$	F: ATCGGTTGGCCTATAAAGTT **hex** R: CACCCCTGTCAGATAGATTGT	*61*	FJ210968
KMm277	$(CA)_{26}$	F: ATAATCAGACAGGCGACAGAT **hex** R: GTGAAAGGTTGGCAAATAATC	*53*	FJ210969
KMm279	$(CA)_{13}CC(CA)_7$	F: CTGGAGAAAATGCAAATGG **hex** R: ACAGTGAGGGGAGAGT TTACA	*61*	FJ210970
KMm291	$(CA)_{10}$	F: AATGCGCACTTTTAAGACCT **6-fam** R: AATTTGCGGTGTGATTTAGAT	*65*	FJ210971
KMm294	$(CA)_{11}GA(CA)_{16}$	F: CGTGAGGGAGTCAAACAC **ned** R: TACAGAGCGGTTCACTAAAAC	*65*	FJ210972
KMm295	$(CA)_{18}$	F: CATCGACGGTCAGTTGC **hex** R: TGACAATGTAAGGGAGGATTT	*53*	FJ210973
KMm34	$(CA)_{24}$	F: AAGTAACTTGCCTTTTGACAC **ned** R: CTACTGGGCACATCATCAT	*53*	FJ210974
KMm37	$(GT)_{34}$	F: AAGGGACTCAAAGTGTCACAG **hex** R: GTTTCATTCCCAGAGACTCAG	*63*	FJ210975
KMm311	$(GT)_{19}$	F: AAGCAGTCTAACCTCGTGTCT **ned** R: TGATTCCTCCTTTTTCTGAGA	*61*	FJ210976
KMm317	$(CA)_{50}$	F: CACGGGCAGTCATGTAGA **6-fam** R: CACCTATAGGTCGGCTTTAGT	*53*	FJ210977
KMm324	$(GT)_{20}$	F: ACTGTGCCACTAATACACTGA **6-fam** R: TGAAGGCCTGTCACTTGT	*65*	FJ210978
KMm326	$(GT)_{25}$	F: CAGACAAGGGTATAGGTGTG **ned** R: TTAATAATGTTGACCTGGTTT	*53*	FJ210979
KMm331	$(CA)_{11}$	F: TTCGGGAATGTTACTGAAAAT **6-fam** R: AGGTTTGATTCGGTGTTTAGA	*53*	FJ210980
KMm338	$(GT)_{12}$	F: GCCATATTTCTCTTACCACTG **ned** R: TGCAAGCCTATACACATAAAG	*61*	FJ210981

*T*a is the optimal annealing temperature.

~rousset/Genepop.htm), which was also used to identify deviations from Hardy–Weinberg equilibrium (HWE; exact tests, 1000 iterations) along with the observed and expected heterozygosities (indicating an excess or deficiency of heterozygotes). FSTAT (ver. 2.9.3.2; Goudet 2001) was used to calculate the inbreeding coefficient (F_{IS}; Weir and Cockerham 1984) per locus and sample and allelic richness (A_R; El Mousadik and Petit 1996), which is suitable for comparing the mean number of alleles among populations irrespective of sample size. ARLEQUIN 3.0 (Excoffier et al. 2005) was used to assess linkage disequilibrium for all pairs of loci using a likelihood-ratio test, whose empirical distribution is obtained by a permutation procedure (Slatkin and Excoffier 1996) and to calculate single-locus and global multilocus values (F_{ST}; 1000 permuta-

tions; Weir and Cockerham 1984). Significance levels were adjusted for multiple tests using the Bonferroni correction technique (Rice 1989).

Results

Microsatellite marker isolation

In total, more than 300 white colonies were obtained from transformation with the Korean mi-iuy croaker $(CA)_n$-enriched genomic DNA library. Of them, 200 colonies were screened by PCR for the presence of a repeat-containing insert. Sequencing of the inserts from these 200 colonies revealed 95 loci containing microsatellite arrays with a minimum of five repeats, corresponding to an enrichment efficiency of about 31.7%. These were primarily 2-bp repeat motifs, some of which were combined with other 2-bp repeat motifs. Primers were designed and tested for 51 loci that exhibited adequately long and unique sequence regions flanking the microsatellite array. Only 22 primer sets successfully yielded variable profiles. The remaining 29 primer sets gave either inconsistent or no PCR products, despite adjusting the dNTP concentrations and using an annealing temperature gradient. With the exception of four loci, KMm241, KMm291, KMm295, and KMm317, which had one allele, all loci were polymorphic and showed differing degrees of variability. The primer sequences, repeat motifs, annealing temperatures, fluorescent labels, and GenBank accession numbers for the 22 new microsatellite loci are summarized in Table 1.

Genetic diversity of the wild and hatchery populations

Samples of 63 wild and 51 hatchery-bred *M.miiuy* collected from Mokpo and Tongyeong, respectively, around the southern coast of Korea were screened for variation at the 18 new polymorphic microsatellite loci. The 18 primer sets yielded variable profiles; 30% of all individuals were retested to ensure that the allele scoring was reproducible.

In total, 403 different alleles were observed over all 18 loci in the samples; the number of alleles varied from four at loci KMm236 and KMm37 to 37 at locus KMm218 (Table 2). Significantly fewer alleles were found in the hatchery-bred population compared with the wild sample (Kruskal–Wallis test, $P < 0.05$). The overall allelic richness varied from 4 to 29.87 (Table 2), with the wild population showing significantly higher allelic richness than the hatchery-bred population (Kruskal–Wallis test, $P < 0.05$).

The MICRO-CHECKER analysis showed that some loci may have been influenced by one or more null alleles in both the wild and hatchery samples; our data showed that loci KMm22, KMm218, KMm244, KMm294, KMm37, and KMm331 in the farmed samples and loci KMm22, KMm216, KMm244, KMm270, KMm34, KMm37, and KMm331in the wild population were affected. Loci KMm22, KMm244, KMm37, and KMm331 appeared to be influenced in both the wild and hatchery samples, indicating that the use of these loci for population genetic analyses that assume HWE may prove to be problematic. Thus, a global multilocus F_{ST} value was estimated with and without these loci. With KMm216, KMm218, KMm270, KMm294, and KMm34, however, factors existed that indicated that these loci were affected by null alleles in only one sample; thus, they were included in further analyses.

We observed no indication that genotyping errors affected allele scoring due to allele dropouts or stuttering at any marker in any of the samples. Samples that failed to amplify after rerunning were not included, making it unlikely that poor DNA quality affected our results.

The observed heterozygosity ranged from 0.412 at locus KMm218 to 0.980 at KMm279, whereas the expected heterozygosity varied from 0.567 at locus KMm236 to 0.968 at KMm326 (Table 2). In terms of heterozygosity, no significant difference was detected between the wild and hatchery samples. Inbreeding coefficients (F_{IS}) varied among markers from -0.340 (KMm236) to 0.494 (KMm218) in the hatchery samples, and from -0.135 (KMm183) to 0.226 (KMm244) in the wild samples. The wild population had more unique alleles than the hatchery population (Table 2).

Significant departures from HWE after Bonferroni correction ($P < 0.003$) were found at all except one locus, KMm277 in the hatchery samples, and at six loci (KMm22, KMm216, KMm244, KMm34, KMm37, KMm331) in the wild samples, indicating that deviations from HWE were due to heterozygote deficiency.

The allele frequencies of the 18 microsatellites in the wild and hatchery samples are depicted in Figure 1. The allele frequency distributions indicated the presence of 40 rare alleles (frequency $< 5\%$) among a total of 129 alleles over all loci (mean 31.01%) in the farmed sample, whereas 332 rare alleles among a total of 386 alleles (mean 86.01%) were observed in the wild sample. Rare alleles were detected at most loci and were not associated with a particular locus in either population. No significant linkage disequilibrium between loci pairs was detected ($P > 0.05$).

Single-locus F_{ST} estimates and global multilocus F_{ST} values were significantly different between the hatchery and wild populations. The global multilocus F_{ST} value, including all loci, was estimated to be 0.064 ($P < 0.001$). When loci KMm22, KMm244, KMm37,

Table 2. Summary statistics for 18 microsatellite loci in the two *Miichthys miiuy* populations.

Population (No)		Microsatellite loci																		
		Kmm183	Kmm22	KMm216	KMm218	KMm236	KMm244	KMm270	KMm274	KMm277	KMm279	KMm294	KMm34	KMm37	KMm311	KMm324	KMm326	KMm331	KMm338	Mean
	F_{ST}	0.089	0.035	0.100	0.093	0.031	0.079	0.061	0.030	0.042	0.068	0.037	0.091	0.132	0.045	0.046	0.077	0.015	0.060	0.064
Mokpo Wild (63)	N_A	10	26	21	37	5	20	12	12	17	27	21	31	25	28	20	32	18	24	21.44
	A_R	8.42	19.17	15.82	29.87	4.41	16.35	11.26	10.57	12.00	21.89	18.53	25.29	20.50	22.85	16.42	27.11	15.19	20.10	17.54
	S	196–220	140–208	228–294	102–204	190–204	220–296	256–294	264–300	224–294	248–328	118–162	58–122	218–290	110–184	178–240	110–190	210–256	124–190	
	F	0.547	0.443	0.302	0.151	0.472	0.208	0.208	0.264	0.575	0.151	0.264	0.123	0.117	0.142	0.170	0.057	0.274	0.113	0.254
	U	6	15	16	31	3	16	6	7	13	22	13	23	22	21	10	24	11	15	15.2
	He	0.666	0.789	0.867	0.960	0.567	0.876	0.878	0.846	0.647	0.934	0.901	0.953	0.960	0.941	0.909	0.968	0.881	0.942	0.860
	H_0	0.755	0.679	0.736	0.906	0.547	0.679	0.698	0.792	0.642	0.906	0.868	0.830	0.831	0.906	0.830	0.962	0.718	0.906	0.788
	F_{IS}	−0.135	0.140	0.153	0.057	0.036	0.226	0.207	0.064	0.009	0.031	0.037	0.130	0.122	0.037	0.088	0.006	0.043	0.039	
		(0.689)	(0.049)	(0.002)	(0.385)	(0.502)	(0.000)	(0.147)	(0.330)	(0.997)	(0.337)	(0.707)	(0.018)	(0.000)	(0.168)	(0.342)	(0.604)	(0.000)	(0.819)	
	P	0.721	0.000	0.000	0.629	0.514	0.000	0.136	0.328	0.989	0.371	0.712	0.000	0.000	0.076	0.187	0.490	0.000	0.673	
Tongyeong	N_A	5	12	5	8	4	7	7	5	5	5	8	9	4	7	12	8	7	11	7.17
Hatchery(51)	A_R	5	12	5	8	4	7	7	5	5	5	8	9	4	7	12	8	7	11	7.17
	S	194–216	140–204	240–250	102–176	198–212	234–286	256–276	266–278	226–288	270–286	120–154	64–114	220–274	111–156	134–222	132–188	222–240	116–156	
	F	0.304	0.304	0.412	0.275	0.510	0.353	0.265	0.324	0.608	0.382	0.275	0.324	0.303	0.255	0.216	0.294	0.284	0.265	0.031
	U	1	1	0	2	2	3	1	0	1	0	0	1	1	0	2	0	0	2	0.9
	He	0.752	0.862	0.661	0.810	0.617	0.776	0.828	0.742	0.588	0.761	0.807	0.820	0.727	0.823	0.886	0.822	0.151	0.806	0.775
	H_o	0.922	0.725	0.745	0.412	0.824	0.490	0.882	0.725	0.647	0.980	0.490	0.745	0.545	0.882	0.922	0.824	0.745	0.863	0.743
	F_{IS}	−0.228	0.160	−0.116	0.494	−0.340	0.370	−0.066	0.023	−0.102	−0.292	0.395	0.092	0.253	−0.072	−0.040	−0.002	0.154	−0.071	
		(0.001)	(0.000)	(0.005)	(0.000)	(0.003)	(0.000)	(0.000)	(0.000)	(0.062)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	
	P	0.001	0.000	0.002	0.000	0.001	0.000	0.000	0.000	0.0078	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Single-locus F_{ST}, number of samples (No), number of alleles per locus (N_A), allellic richness (A_R), size in bp of alleles (S), frequency (F) of the most common allele, number of unique alleles (U), expected heterozygosity (*He*), observed heterozygosity (*Ho*), inbreeding coefficient (F_{IS}), and probability of significant deviation from Hardy–Weinberg equibrium after Bonferroni correction (*P*, initial $\alpha = 0.05/18 = 0.003$) are given for each population and locus. Calculations assume that individuals with one microsatellite band are homozygous for the allele. Number in parenthesis below F_{IS} indicates the probability of significant heterozygosity excess or deficit.

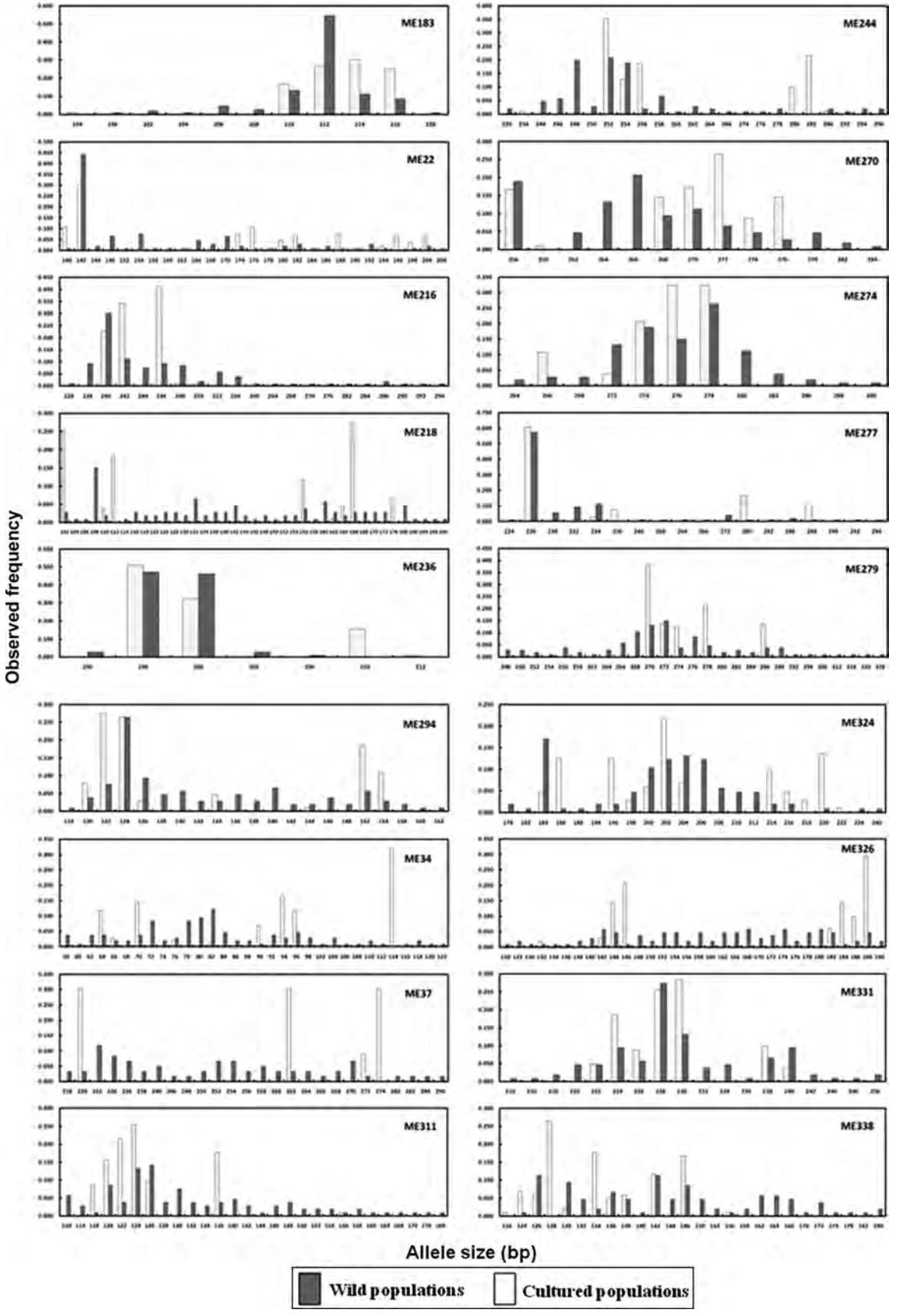

Figure 1. Allele size frequency distributions of the 18 microsatellite loci of *Miichthys miiuy* used in this study.

and KMm331 were excluded, the global multilocus F_{ST} was estimated to be 0.058 ($P < 0.001$).

Discussion

Overexploitation of *M. miiuy* will eventually lead to the loss of genetic diversity. Thus, understanding the genetic diversity of mi-iuy croaker populations is important for the sustainable fishery management of the species. MS DNA loci are expected to provide an invaluable tool for this purpose because their highly polymorphic characteristics have great potential as genetic tags for use in aquaculture. To this end, we have identified and characterized microsatellite markers for the Korean mi-iuy croaker, *M. miiuy*.

Traditionally, the isolation of MS DNA loci has relied on the screening of genomic libraries using repetitive probes and sequencing of positive clones to develop locus-specific primers, which is obviously a tedious task, but can result in numerous MS DNA loci being obtained. Magnetic bead-based enrichment is a popular method for constructing MS-enriched libraries. The types and ratios of biotin-labeled probes and positive clone selection strategy may affect the cloning success and enrichment efficiency. In this study, we created microsatellite libraries enriched for CA repeat sequences by following the protocol of Hamilton et al. (1999), using the modifications described by Gardner et al. (1999) and Carleton et al. (2002). Of the positive clones obtained, about 31.7% contained microsatellite repeats (95/300). This number is comparable with that reported for Japanese Spanish mackerel (34%; Yokoyama et al. 2006) and Korean rockfish *Sebastes schlegeli* (32%; An et al. 2009), but lower than that for tilapia (96%; Carleton et al. 2002) using several enriched libraries differing in the selected size of the restriction-digested genomic DNA. The differences in enrichment efficiency may be a result of the use of various biotin-labeled oligonucleotide probes and the proper ratio, rather than different absolute numbers of repeats in each genome.

The high level of genetic diversity (mean heterozygosity = 0.86; mean allelic number = 17.54) in the wild population in this study is similar to that reported in most other marine fishes (DeWoody and Avise 2000).

After sequential Bonferroni correction for multiple tests, significant deviations from HWE in the direction of heterozygote deficiency were detected at six of the 18 microsatellite loci in the wild population samples. Previously, heterozygote deficiency has been reported based on microsatellite data from populations of other marine fish (Sekino et al. 2002; Yue et al. 2004). Generally, heterozygote deficiency increases due to factors such as inbreeding, substructuring of the population sample, or the presence of null alleles. From the results of previous studies, null alleles, a locus-dependent effect found frequently at MS DNA loci, are the most likely cause of the heterozygote deficiency in HWE tests. Indeed, our MICRO-CHECKER analysis revealed the presence of null alleles at those six loci, with a significant heterozygote deficit. Thus, most deviations from HWE in the wild population might have been due to the presence of null alleles resulting from base substitutions or deletions at the PCR priming sites in the flanking region of the microsatellites. However, significant deviations from HWE were found at all except one locus, KMm277, in the hatchery samples. Nine of the 17 significant HWE deviations in hatchery samples revealed heterozygote excess. The sampling bias of alleles, resulting from a small number of parents and differences in allele frequencies between the sexes, sometimes leads to a reduction of homozygotes when the population has experienced a recent reduction in size (Spencer et al. 2000; Launey et al. 2001). The overdominance phenomenon, which causes a lower survival of homozygotes, might be one factor for heterozygote excess (Fujio et al. 1985).

Considering that this study was limited by the number of populations screened, the genetic diversity statistics for each population, as well as the HW disequilibrium at loci KMm216 and KMm34 observed in wild samples, can perhaps be explained by data from additional populations, which may provide more precise estimates for the genetic characterization of the MS loci used. Thus, our results should be interpreted with some caution. Further study is required to assess the genetic resources of wild populations and the influence of aquaculture on the genetic structure of this important fishery species.

The wild and hatchery populations showed significantly different single-locus F_{ST} estimates and global multilocus F_{ST} values. The significant F_{ST} estimates indicate the presence of genetic differentiation between the populations. This difference was probably a result of reduced genetic variation. If the number of effective breeders is not maintained, the continued reproduction of the hatchery-bred strain might lead to reduced genetic variability. Several studies have reported a loss of genetic variation at MS loci in hatchery populations and reduced fitness in hatchery-bred individuals when exposed to natural environments (Hansen 2002; Li et al. 2004). Reduced genetic variation can result in reduced performance in aquaculture because this is the source of variation for important traits, such as growth rate and disease resistance (Allendorf and Phelps 1980; Vuorinen 1984). In fact, the loss of alleles is more important than a change in allele frequencies because the latter can be changed again by random drift,

whereas there is no way to recover a lost allele. For the proper management of a commercial breeding program, monitoring the genetic structure and diversity must be considered in addition to biological, ecological, and fishery factors. Thus, samples from the wild population should be taken and analyzed with genetic markers prior to being used as broodstock. A sample of the hatchery-reared fish should then be taken for genetic analysis. This information will be useful in evaluating the feasibility of a commercial breeding program to maintain the genetic diversity of wild populations, as well as improving hatchery management in subsequent seasons.

In summary, we report that the newly developed microsatellite markers can detect population structure in *M.miiuy*. These markers may be useful for future population genetic structure studies, including the monitoring of changes in genetic variation within stocks in a commercial breeding program, assignment tests, pedigree analysis, and mapping.

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