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Microsatellite analysis as a tool for discriminating an interfamily hybrid between olive flounder and starry flounder

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ABSTRACT. An interspecific artificial hybrid was produced between two economically important aquaculture flatfish: olive flounder (*Paralichthys olivaceus*) and starry flounder (*P. stellatus*). This hybrid displays the rapid growth characteristic of the former and tolerance to low temperatures and low salinity of the latter, but the genetics of inheritance in this hybrid have not been elucidated. Polymorphic microsatellite markers developed for *P. olivaceus* and *P. stellatus* were tested to determine if these markers can be used for analysis of parentage and genetic inheritance. Multiplex PCR using two primer sets that were specific to each species produced PCR products of different sizes; these could be used for the identification of interspecific hybrids. Among the 192 primers derived from olive flounder, 25.5% of the primer sets successfully amplified genomic DNA from starry flounder, and 23% of the 56 primer sets originating from starry flounder amplified DNA from olive flounder. Analysis of genetic inheritance in the hybrid using seven of the 62 microsatellite markers common to both species demonstrated classic Mendelian inheritance of these markers in the hybrid progeny, with the exception of one locus identified as a null allele in the hybrid.

These results demonstrate that cross-specific microsatellite markers can be used tools for parentage analysis of hybrid flatfish, for mapping quantitative trait loci, for marker-assisted selective breeding, and for studies of the evolution of fish.

Key words: Hybrid; Microsatellite; Olive flounder; Starry flounder

INTRODUCTION

Hybridization is more common in fish than in any other vertebrate because of their external fertilization and similar mating behaviors (Leary et al., 1995). In addition to hybridization in natural ecosystems, usually between native and nonnative taxa, artificially induced hybridization has been employed as a tool to improve aquaculture productivity through heterosis in fish (Moav, 1979; Ihssen et al., 1988; Wohlfarth, 1993).

The olive flounder *Paralichthys olivaceus* (Temminck and Schlegel) is one of the most important fishery and aquaculture species in Korea, Japan, and China. The starry flounder *P. stellatus* (Pallas), another flatfish in the same order (Pleuronectiformes), is a native fish in the vicinity of the Korean Peninsula. It has a slow growth rate but high tolerance to low temperatures and low salinity. Therefore, a hybrid between *P. olivaceus* and *P. stellatus* was produced to enhance growth and tolerance to environmental stresses. Aquafarming of the hybrid is underway in Korea (Nam et al., 2008). Although the hybrid fish displayed the intended phenotypes, their similar morphology made distinguishing the hybrid progeny from their parents difficult, especially in their early stages.

Several molecular markers such as alloenzymes (Berrebi et al., 2000), mitochondrial DNA (Snoj et al., 2000), microsatellites (Fumagalli et al., 2002), random amplified polymorphic DNA (RAPD; Jug et al., 2004), and single nucleotide polymorphisms (Harwood and Phillips, 2011) have been used for the discrimination of hybrids and parentage analysis in fish. Although each method has advantages and drawbacks in their application, the microsatellite markers are highly useful because they are abundant, evenly distributed, and highly polymorphic. They are also short in length, facilitate genotyping by polymerase chain reaction (PCR) and are codominant markers that allow the generation of maximum genetic information. Polymorphic microsatellite markers have been developed in both *P. olivaceus* and *P. stellatus* and have been applied to parentage relationships, monitoring populations, and linkage mapping of these fish (Jarne and Lagoda, 1996; Li et al., 2004; Kang et al., 2008).

In this study, polymorphic microsatellite markers developed for *P. olivaceus* and *P. stellatus* were tested against one another to identify a possible application of these markers in parentage analysis of the hybrid, which would provide valuable information in fish breeding programs and facilitate understanding the molecular dynamics in the hybridization of two fish species from different families.

MATERIAL AND METHODS

Production of hybrids

An F_1 hybrid family was produced by controlled interspecific hybridization between one female olive flounder and one male starry flounder. The male parent was from wild stock and the female parent was from cultured stock, both collected and maintained at the Uljin

Hatchery Center, National Fisheries Research & Development Institute, Korea. The hybrids were raised by running water culture before analysis.

Sample collection and DNA extraction

A total of 48 progeny (6 months of age, average weight of 8.5 g) and their two parents were used for the genetic inheritance analysis. Ten of each olive flounder, starry flounder and their hybrid progeny kept at the Uljin Hatchery Center were used for species identification and parentage confirmation of hybrids by multiplex PCR using species-specific primer sets. Small pieces of fin tissue were cut from individuals and preserved in 100% ethanol. Total DNA was isolated from each sample using a MagExtractor MFX-6100 automated DNA extraction system (Toyobo, Osaka, Japan). The extracted genomic DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and stored at -20°C until microsatellite genotyping analysis.

PCR amplification and genotyping

In total, 192 olive flounder microsatellite markers used in this study were taken from a previous study for the construction of an olive flounder linkage map (Kang et al., 2008). All 56 microsatellite markers for starry flounder were taken from the GenBank/EMBL/DDBJ database with the prefix Ksf. For multiplex PCR, the 5'-end of each forward primer was labeled with one of three fluorescent dyes: 6-FAM, HEX, or NED (Applied Biosystems, Foster City, CA, USA). PCR for the amplification of microsatellite loci was performed in a 10-µL reaction volume containing 1X ExTaq buffer, 10-50 ng template DNA, 0.2 mM dNTPs, 0.5 µM of each primer, and 0.25 U *Taq* DNA polymerase (Takara, Shiga, Japan), using an RTC 200 thermocycler (MJ Research, Waltham, MA, USA). The PCR conditions were as follows: initial denaturation at 95°C for 11 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at optimum temperature for each primer set for 1 min, and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. A 1-µL aliquot PCR product was mixed with a genotyping reaction mixture containing formamide and a size standard using GeneScan 400HD ROX (Applied Biosystems) and electrophoresed with an ABI 3130 DNA sequencer (Applied Biosystems). The fragment lengths of the PCR products were determined using the GeneMapper ver. 4.0 software (Applied Biosystems). Differences between observed genotypes and Mendelian expectations were assessed using the χ^2 test. Multiplex PCR with olive flounder-specific *Kop60* marker and starry flounder-specific *Ksf5* marker was conducted as above and the PCR product was electrophoresed on a 1.5% agarose gel.

RESULTS

Common microsatellite markers

In total, 248 microsatellite markers, including 192 markers derived from olive flounder and 56 markers from starry flounder, were cross-checked between one another by classical agarose gel electrophoresis. Among the 192 primers derived from olive flounder, 49 (25.5%) primer sets successfully amplified genomic DNA from starry flounder. Similarly, 13 (23.2%) of the 56 primer sets originating from starry flounder amplifed DNA from olive flounder. The information for these primers is shown in Table 1.

Table 1. Information of microsatellite markers common in olive flounder and starry flounder.

Locus	Source	Primer sequence (5'→3')	Ta (°C)	PCR size (bp)	Accession No.
Poli2TUF	*Paralichthys olivaceus*	ACAATAGGATGCAGCTGCCT AAGCGCAAATTGTTATTCCG	62	108-126	AB037978
Poli11TUF	*Paralichthys olivaceus*	ATGAAAACCACCAAGAATCCC GGCGCATTTGGTAGTTTGTT	62	89-132	AB037981
Poli18TUF	*Paralichthys olivaceus*	CACGCACACACAAGCTCC CGTGGGGTGAGGTTATGG	65	123-147	AB037983
Poli29TUF	*Paralichthys olivaceus*	AACTCCACAAAAAGCTGATCACAGC GCCATTCTACAAGCAGCTGCACTAT	65	139-147	AB037987
Poli38TUF	*Paralichthys olivaceus*	CTTACACACAAAGCACAGCCA CCAGTCTGACATGAAGCGG	65	97-132	AB459378
Poli105TUF	*Paralichthys olivaceus*	TGTCTGTAAATACACCTCCTCTCCCA TCATCTCCCTGCTCTGACTCAGG	52	93-105	AB459390
Poli106TUF	*Paralichthys olivaceus*	CTGGCTTTTTAAGGAGAGCCACACT GATCGACCTTGATGAGACTCACAAA	55	117-124	AB459391
Poli115TUF	*Paralichthys olivaceus*	GATGTAGGTCACACACTGAGGCTGA CCTTTGCATTTGTCTACGCAAGC	62	152-201	AB459399
Poli124TUF	*Paralichthys olivaceus*	AGGAGCATGGCAATGTGAGACAC GTACACTGTGAATCCAAGCCCAGTT	62	94-110	AB459402
Poli129TUF	*Paralichthys olivaceus*	TTGCTGACTTCCTCTGTTTACTCCC ACAGCTGCTCTGTGTGTATGTTTGC	62	82-112	AB459406
Poli136TUF	*Paralichthys olivaceus*	CTGGTTGGAGTGGATGTAGGCTG GTGAGACAGGTGCTGAGTCTTCTCC	55	124-143	AB459411
Poli138TUF	*Paralichthys olivaceus*	CCCGTCTTTAGTTTTTTCTTCTTGACC CGGGAGGAAAGTCAGGTCATTAACT	60	118-124	AB459412
Poli145TUF	*Paralichthys olivaceus*	TGTTGCTCTGACACAGAGGAATGTC GAATGTATTTCCCTGTTTGGCATGA	60	122-149	AB459419
Poli149TUF	*Paralichthys olivaceus*	CGTGTACAGTACACCTCCCAACAGA GCTTGCGCCTGACAAACTGATATAA	60	119-156	AB459423
Poli162TUF	*Paralichthys olivaceus*	GTCCGTCAAATGGATTCAATGCTAA CATGTCTCTGGACTTCACACAGGC	55	96-128	AB459434
Poli163TUF	*Paralichthys olivaceus*	ATCCACATCCCCTTTAGGCTAGTCC ATGGCCTTGTGTTTGTTTCTGTTTG	60	145-185	AB459435
Poli174TUF	*Paralichthys olivaceus*	TAGAAACTGGCCTTCATGGTGTCTC ATGTCAGAGTTTGAAAGCAGCAACC	62	127-164	AB459445
Poli177TUF	*Paralichthys olivaceus*	CTCGGCCTGTTTAATCTAATTTTTGC AAGGTCACTGAGACATGAGAGGCAG	60	121-138	AB459448
Poli183TUF	*Paralichthys olivaceus*	CAAGGGCACCTTGTCTTAAAGGAAA TTGATTGATAGTTTGACTGAGTCAGCA	58	129-143	AB459454
Poli190TUF	*Paralichthys olivaceus*	GAGTATACCCACTTCTCCAGGCACC AAGAAACCAATCCACCGTTCACTG	62	136-156	AB459460
Poli194TUF	*Paralichthys olivaceus*	TGCAGCTGACTAATCCACTGCAG AGTGTGTGTCTGTCCACTCTTGTGC	65	126-143	AB459464
Poli198TUF	*Paralichthys olivaceus*	TGGCAGTCTTATCAGCCTCTGCTAC GGTGTCTGATGCTATCTCCACAGCT	62	128-218	AB459467
Poli200TUF	*Paralichthys olivaceus*	CTTGCTGACTTCCTCTGTTTACTCCC ACAGCTGCTCTGTGTGTATGTTTGC	62	83-113	AB086615
Poli9-8TUF	*Paralichthys olivaceus*	GAGAGACAGAAGGTCGTCAACGGTA ACAAAGACCACGATGCAAAGTGAC	62	139-141	AB037989
Poli13-2TUF	*Paralichthys olivaceus*	TCATCCCATTAAAGCATAGCG ATCTCACAGCATCACTTGATGG	60	95-123	AB459357
Poli16-24TUF	*Paralichthys olivaceus*	GCCGTCAACACAGAACTGAG TCTGAGAGATGATGACGCATC	55	129-146	AB459363
Poli16-76TUF	*Paralichthys olivaceus*	GCAGTGAGTGGACTTCAGGTGCTAT TATAAGGTGGGAGAGAGGAAGGTGG	65	99-105	AB459367
Poli18-42TUF	*Paralichthys olivaceus*	GATCTCTGGAGGAGGAGGACGAC CAGATAACATGCAGGTCACATCTGG	55	144-169	AB459373
Poli18-44TUF	*Paralichthys olivaceus*	ATCCACTGAAGATAGTTTGGTTTGGG CAGTCATCATGTTCCGAAACCATC	55	129-148	AB459374
KOP3	*Paralichthys olivaceus*	AGAGGATATCGAGGGGAGG CAGCAGTAGCCGATCTTAGTG	65	114-116	AY328959
KOP18	*Paralichthys olivaceus*	GAAGGATCTGGACTCATGGTGAC CAGCAGCAAAGGCAGAAAGAG	67	199-237	AY328973

Continued on next page

Table 1. Continued.

Locus	Source	Primer sequence (5'→3')	Ta (°C)	PCR size (bp)	Accession No.
KOP19	*Paralichthys olivaceus*	TCCGGTTCAGGAGAGTTCAATG GGTGGTTTTGATCAGTCCATATG	65	184-239	AY328974
KOP23	*Paralichthys olivaceus*	TCGATGAGTGTCCTCGCAACTA ACCGCAGTCTGCAGTTCCTCT	65	161-194	AY328978
KOP24	*Paralichthys olivaceus*	GCACGCTACCTGACTGAGAC GGCTGGATTCTTCTGCTTC	63	100-108	AY328979
KOP26	*Paralichthys olivaceus*	CAGTAAAACAGTCCCTCCTGAAC GGAGTCTGGAACCAAATGTCTG	60	181-201	AY328981
KOP32	*Paralichthys olivaceus*	TCAAACACTCATCCGTCTTC GTTTCTCATGACTGGCTTGTAG	60	183-197	EU307225
KOP44	*Paralichthys olivaceus*	GATTCTCAACGGCAGACCATT GATCCCACCTTAAAAGTCAG	56	206-214	EU307234
KOP46	*Paralichthys olivaceus*	AGAGTAACTACAGGAACTGCC CAGTGCCCAACCTCTG	56	140-160	EU307237
KOP57	*Paralichthys olivaceus*	GTTCATGTTTGACGGTCCTCG GGGATTTGAAAGCGGGATTAGG	56	209-215	EU307247
KOP68	*Paralichthys olivaceus*	AGGTCAGGGTCACTCGTG TGACAAGAGGAATCATCACAA	56	131-134	EU307254
Poli5MHFS	*Paralichthys olivaceus*	AAAGCAGAAGGGTCAAGC CAAAGATCGAGGGTCAGC	60	110-139	AB459314
Poli12MHFS	*Paralichthys olivaceus*	CAGTGCCTAAACCAGTGT TGTGCTACCGTGAATAAT	60	165-205	AB459319
Poli14MHFS	*Paralichthys olivaceus*	CCAGCCAAAACAAAACCATA AATAACAAAGAACGGACAGC	60	113-178	AB459321
Poli31MHFS	*Paralichthys olivaceus*	GGAGAGTTTGCTGAGTCAAC TGTAAGCTGAGGAAAGAAAT	60	115-135	AB459329
Poli32MHFS	*Paralichthys olivaceus*	GCTGGGTTGGTGGGAGTTATGG AAGGTTAGGTTACGGTTAGACA	60	134-155	AB459330
Poli38MHFS	*Paralichthys olivaceus*	CAGTTGAAGGGAGTGATGTC GGAAGGAAATAGTTAGAGTG	60	183-260	AB459332
Poli39MHFS	*Paralichthys olivaceus*	GGCCTTGTTGTTGTCTGTGA ACCGAATGTGAATCTGAAAA	60	167-189	AB459333
Poli66MHFS	*Paralichthys olivaceus*	TCTACCAAACTCTCAATCCT TCACTTCACTTATCCCACAG	60	194-207	AB459335
Poli92MHFS	*Paralichthys olivaceus*	ATCACTGTTTCATTAGGG CTGGACGCATTCTTTGTA	60	106-133	AB459341
Ksf1	*Platichthys stellatus*	CGCCACATAATAACAGCA AAGACATGACTGGGAGAA	54	221-229	JF913209
Ksf13	*Platichthys stellatus*	AGAAGAGCTCTGTGACTC GAGACACACTGACAAACC	54	133-169	JF913221
Ksf14	*Platichthys stellatus*	ACAGCAGCCGATAACATGAG CGTCCCCTTATGATGAGACAAC	58	233-275	JF913222
Ksf15	*Platichthys stellatus*	GGAAGAGACAGTTCACCA CTCAGCAGTCTTCAAACC	54	251-263	JF913223
Ksf25	*Platichthys stellatus*	GCCTCTTAGGTAAGACATGCGA GCTGTCACCCTGTTCAACCA	58	129-137	JF913233
Ksf26	*Platichthys stellatus*	CAGGGACATGAAAAGGTTG GAGCATGTCAGAAAGATGG	56	129-137	JF913234
Ksf29	*Platichthys stellatus*	GAGACTCAGATCGCAGTCCA CCCCTGAGATCAAGGGTGTT	58	138-148	JF913237
Ksf33	*Platichthys stellatus*	CAGGCAGATGTCTATGGGATGA CTTACCGAATGGGACAGCAAAC	56	188-198	JF913241
Ksf35	*Platichthys stellatus*	GCAATGAAACGTGTTCTG GGCTGTGTTGATGATCTC	54	104-140	JF913243
Ksf46	*Platichthys stellatus*	CCTGCGTTTGACTTCAACTG AACCACAGAAGCTGTTCTCC	58	156-258	JF913254
Ksf47	*Platichthys stellatus*	ATGCAACAGCTCCGAGGCAA GCTGACGTGAAGACTGGGACAA	58	132-176	JF913255
Ksf54	*Platichthys stellatus*	AGACGGTTCACTCTGAAG GTAGCCGTACAAACATGG	54	208-216	JF913262
Ksf55	*Platichthys stellatus*	AAGCCCTCATTCTTCGAAGCTC AATAAGGAGCCGGTGCTCAA	58	236-248	JF913263

Ta = annealing temperature; PCR = polymerase chain reaction.

Identification and parentage confirmation of hybrids

Multiplex PCR using two primer sets that are specific to each species and produce PCR products of different sizes could be used for the identification of hybrids. For example, the primer set for the olive flounder-specific *Kop60* marker and starry flounder-specific *Ksf5* marker produced corresponding PCR products in each parent and two PCR products from the hybrid of the two species (Figure 1).

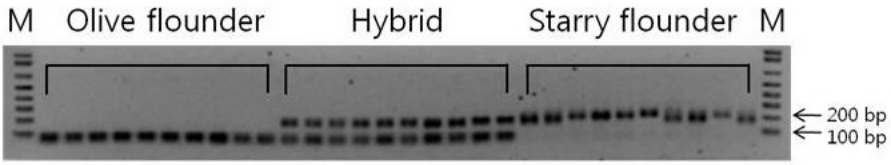

Figure 1. Amplification profile of olive flounder, starry flounder and their hybrid in multiplex PCR with primer sets for olive flounder-specific *Kop60* and starry flounder-specific *Ksf5* markers.

In contrast, the primer set for the markers common to two species could be used for the analysis of genetic inheritance. Among the 62 microsatellite markers common to both species, seven markers (*Kop24*, *Kop68*, *Poli108TUF*, *Poli11TUF*, *Poli172TUF*, *Poli13-2TUF*, and *Poli131TUF*) showing polymorphism in the parent genotype were selected for genetic inheritance analysis. The inheritance of each microsatellite in the interfamily hybrids was analyzed, and two examples shown in Figure 2 demonstrate classic Mendelian inheritance of the markers in the hybrid progeny. Table 2 shows the genotype of the female olive flounder and male starry flounder, the expected separation of genotype, and observed genotype ratio in the hybrid. The genotype analysis showed a typical Mendelian ratio of 1:1:1:1 at most loci with the exception of the *Poi172TUF* locus.

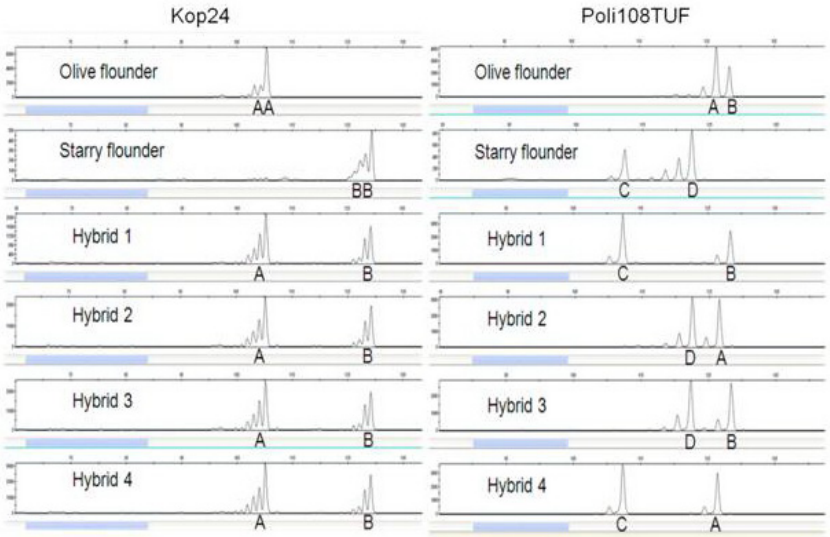

Figure 2. Genotyping of parental olive flounder, starry flounder and their hybrid using microsatellite markers *Kop24* and *Poli108TUF*, which are common to both species.

Table 2. Genetic inheritanc analysis of hybrid from olive flounder and starry flound with seven common microsatellite loci.

Marker locus	Parent genotype		Hybrid genotype		P value
	Olive flounder	Starry flounder	Expected pattern	Observed ratio	
Kop24	AA (110/110)	BB (129/129)	AB	48	1
Poli108TUF	AB (126/128)	CD (112/122)	AC:AD:BC:BD	15:13:9:11	0.644
Kop68	AB (132/134)	CC (144/144)	AC:BC	25:23	0.773
Poli11TUF	AB (111/117)	CC (105/105)	AC:BC	28:20	0.248
Poli172TUF	AA (123/123)	BB (113/113)	AB	0 (AA = 48)	0
Poli13-2TUF	AB (96 / 98)	CC (112/112)	AC:BC	25:23	0.773
Poli131TUF	AA (107/107)	BC (101/103)	AB:AC	22:26	0.564

DISCUSSION

Heterosis resulting from crosses between different species shows phenotypic superiority of a hybrid over its parents with respect to traits such as growth rate, reproductive success, and yield, probably by some non-mutually exclusive mechanisms such as dominance complementation, overdominance, and epistasis (Duvick, 1999). Although hybridization is a new technique in aquaculture compared to animal husbandry and agriculture, several biological characteristics of fish, such as high fecundity, external fertilization, and relative simplicity of obtaining viable and fertile hybrids, have promoted the hybridization of aquaculture fish. Hybrids have been successfully produced and commercially used in sturgeon, white fish, tilapia, catfish, and various cyprinids (Bartley et al., 2000).

One interesting feature in the production of the hybrid between *P. olivaceus* and *P. stellatus* is that they belong to different families of the order Pleuronectiformes; *P. olivaceus* belongs to the family Paralichthyidae, and *P. stellatus* belongs to the Pleuronectidae. Although hybrid fish have been produced by bigeneric crossing, such as between the two sturgeons *Huso huso* and *Acipenser ruthenus* and between silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), most hybrid fish that have been developed to date are intraspecific or interspecific (Bartley et al., 2000). Although *P. olivaceus* and *P. stellatus* belong to different families, sequence analysis of the mitochondrial 16S rDNA showed that these two families are very close to each other on the phylogenetic tree, which could explain the feasibility of hybrid production (Pardo et al., 2005; Azevedo et al., 2008). The hybrid between the female *P. olivaceus* and male *P. stellatus* showed more morphological similarity to *P. stellatus*; growth was improved, but the hybrid was not fertile (Nam et al., 2008).

Accurate identification and screening of hybrids is very important in breeding programs and in the analysis of hybrid invasion in natural environments, which is time-consuming and difficult in some species. Molecular markers, such as RAPD markers and microsatellite markers, have been used for the identification of hybrids in plants (Asif et al., 2009; Conceição et al., 2011) and fish (Wenburg et al., 1998). One limitation in the application of microsatellite markers for hybrid identification is an overlapping allele size. In our study, we used species-specific primer sets for identifying the hybrid by multiplex PCR. In addition to the species-specific fixed markers of different sizes, a primer set common to both parents but that produced allele products of different size ranges in different parents, could be used for both hybrid identification and inheritance analysis.

Microsatellite markers can be developed by many methods, for example, comparing

massive sequences such as the EST sequence, cloning random segments of DNA from the focal species followed by screening with fluorescent-labeled oligonucleotide sequences that will hybridize to a microsatellite repeat, or by enrichment of the microsatellite with oligonucleotides complementary to repeats (Kaukinen et al., 2004). Despite these advances, the development of correctly functioning primers is often a tedious and costly process. One way to solve this problem is to apply microsatellite markers developed for a particular species to closely related species. Liu et al. (1999) showed that 29 of 32 primer sets designed from channel catfish (*Ictalurus punctatus*) successfully amplified genomic DNA from blue catfish (*I. furcatus*). They also showed that 14 of them amplified genomic DNA from flathead catfish (*Pylodictis olivaris*) and 13 amplified microsatellites in white catfish (*Ameiurus catus*), which suggests the possible construction of a unified catfish map using conserved polymorphic markers. Wu et al. (1999) also demonstrated that polymorphic microsatellite markers could be amplified from different species of cichlid fish belonging to the same family (Cichlidae). However, the percentage of loci that successfully amplify may decrease with increasing genetic distance (Jarne and Lagoda, 1996). For example, among 64 microsatellite markers derived from olive flounder, 16 (25.0%) and 17 (26.5%) could amplify the corresponding DNA fragment from barfin flounder (*Verasper moseri*) and spotted halilbut (*Verasper variegatus*), respectively (Ma and Chen, 2011). In our experiment, 49 (25.5%) of 192 markers derived from olive flounder successfully amplified DNA fragments from *P. stellatus*. Considering that *P. stellatus* belongs to the same family (Paralichthyidae) of the order Pleuronectiformes, the value is higher than that of the previous results, probably because more markers derived from olive flounder were used. In fact, 13 (23.2%) of the 56 markers derived from starry flounder amplified the target DNA from olive flounder; this percentage is very close to that of Ma and Chen (2011).

In addition to the search for microsatellite markers that can undergo cross-species amplification of polymorphic DNA fragments, selected markers were used to analyze Mendelian inheritance of genotypes in the hybrid of two species. Among the seven microsatellite markers used, six showed Mendelian inheritance, suggesting that cross-specific microsatellite markers are useful tools for the parentage analysis of hybrid organisms.

Many useful characteristics, such as rapid growth and resistance to disease and environmental stresses, tended to be quantitative traits, and the availability of conserved microsatellite markers will be helpful in mapping those quantitative trait loci, marker-assisted selective breeding, and understanding the evolution of aquatic organisms.

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