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Genetic characterization, morphometrics and gonad development of induced interspecific hybrids between yellowtail flounder, *Pleuronectes ferrugineus* (Storer) and winter flounder, *Pleuronectes americanus* (Walbaum)

I-S Park[1], Y K Nam[2], S E Douglas[3], S C Johnson[3] & D S Kim[2]
[1]Division of Ocean Science, Korea Maritime University, Pusan 606-791, South Korea
[2]Department of Aquaculture, Pukyong National University, Pusan 608-737, South Korea
[3]Institute for Marine Biosciences, National Research Council, Halifax, Nova Scotia B3H 3Z1, Canada

Correspondence: Dr Dong Soo Kim, Department of Aquaculture, Pukyong National University, Pusan 608-737, Korea.
E-mail: dongskim@nuri.net

Abstract

Viable interspecific hybrids between yellowtail flounder (*Pleuronectes ferrugineus*, Storer) and winter flounder (*Pleuronectes americanus*, Walbaum) were produced by artificial insemination of yellowtail flounder eggs with winter flounder sperm. However, mean fertilization rate, hatching success and early survival up to 3 weeks post hatch were significantly lower than those of parental pure cross controls ($P < 0.01$). Overall, cytogenetic traits (karyological analysis and estimation of cellular DNA contents using flow cytometry) of hybrid flounder were intermediate between the two parental species. Microsatellite assay was used to distinguish the parental genomes in the hybrids; in most cases, one allele was specific to each of the parents. Morphometrics assessed by body proportions indicated that hybrids generally displayed a morphology intermediate between the maternal and paternal species. Interspecific hybrids exhibited abnormal and retarded gonad development in both sexes based on histological analysis of gonads from adult fish. The sterility of the hybrids presents a significant advantage for their use in aquaculture, as potential escapees would not be capable of reproducing in the wild and contaminating natural stocks.

Introduction

Flounders have been important species in South Korean marine culture since the mid 1980s. However, the productivity of olive flounder (*Paralichthys olivaceus*, Temminck & Schlegel), the most popular cultured species in Korea, has declined gradually during the last decade, due to pollution of coastal areas, diseases and inbreeding depression. Fish farmers and aquaculturists in South Korea are faced with the challenge of finding alternative flounder species that can be grown in existing culture facilities used in the production of olive flounder.

Yellowtail flounder *Pleuronectes ferrugineus*, (Storer) and winter flounder, *Pleuronectes americanus*, (Walbaum) are highly valued commercial and recreational species along the east coast of North America and especially in Atlantic Canada (Brown, Helm & Moir 1995). These two species have also been identified as potential aquaculture candidates highly suitable for markets in Asia including South Korea (Litvak 1999). The merits of yellowtail flounder include good flesh colour and quality, whereas winter flounder are extremely hardy and possess excellent tolerance to a wide range of salinity and temperature. These traits

make them excellent candidates for culture in a number of coastal sites in South Korea.

Both of these species are exotic to the Pacific Ocean and the introduction of these species into South Korea raises concerns about ecological risks particularly with respect to genetic introgression or contamination of the local gene pool by unwanted reproduction of inadvertently escaped or released fish with natural stocks (Kim, Jo & Lee 1994; Beardmore, Mair & Lewis 1997; Saegrov, Hindar, Kalas & Lura 1997; Gross 1998). Methodologies for reproductive containment would be required to reduce the potential for environmental impact resulting from fish escapes.

Hybridization, a method for combining desirable geno- and phenotypes from two different species, can be used for generating sterile fish. Interspecific and intergeneric hybrids may be functionally sterile owing to genetic incompatibility; however, several hybrids showing fertile gonad development have been obtained (see Chevassus 1983; Tave 1993). Further, hybrid fish may offer opportunities for improving production characteristics such as flesh quality, disease resistance, morphology and growth.

This study was carried out as a part of an international co-operative project between Pukyong National University, South Korea and Institute for Marine Biosciences, NRC, Canada, to develop a commercially desirable but sterile flounder population by hybridization between yellowtail and winter flounders, and to examine the potential for their use in South Korean aquaculture. The objectives of this study were (1) to produce viable interspecific hybrids between yellowtail flounder and winter flounder; (2) to characterize the genetic and morphometric traits of induced hybrids; and (3) to determine whether the hybrids display sterile gonad development.

Materials and methods

Generation of hybrids

Wild broodstock were obtained from Atlantic Canadian waters in April 1999. Fish were maintained in ambient seawater and under ambient photoperiod at the Institute for Marine Biosciences (IMB) research station at Sandy Cove, Nova Scotia. A pool of eggs for each species was obtained by stripping eggs from three mature yellowtail flounder and four mature winter flounder. Milt was also collected from each species, pooled within species, diluted 40 times with 0.85% saline and then kept on ice until use. The pool of yellowtail flounder eggs was divided into six aliquots containing approximately 5000 eggs each. Three aliquots were fertilized with winter flounder sperm using a wet method to generate the hybrids. Each aliquot of the eggs was mixed with 2 mL of diluted sperm and activated by an addition of 10 °C seawater. The fertilized eggs were rinsed once with clean seawater. The remaining three aliquots were fertilized with pooled yellowtail flounder sperm to produce pure cross controls of yellowtail flounder. Winter flounder controls were also prepared using three aliquots of approximately 5000 winter flounder eggs and pooled winter flounder sperm. There were nine experimental groups in total, with three replicates per genotype. After fertilization, the eggs were rinsed in clean seawater and maintained at 10 ± 0.5 °C until hatch. Fertilization rate was determined at 3 h post fertilization by examining a random sample of > 100 eggs per replicate for cleavage. Hatching success and survival up to 3 weeks post hatch was also determined. After yolk sac absorption, fish in the replicates within each genotype were pooled and transferred into individual 100-L tanks. Larvae were reared at approximately 15 °C and fed with rotifers and/or brine shrimp until 1 month of age, at which time they were weaned onto a commercial fish feed. After weaning, fish were maintained in separate groups at ambient temperature for up to 18 months before sampling.

Analysis of karyotype and cellular DNA content of induced hybrids

When the fish were 3 months old, 22 fish were randomly chosen from each genotype for chromosome analysis. Metaphase spreads were obtained from kidney cells as described by Kim, Nam & Park (1995) and at least 12 countable metaphase spreads per fish were examined. Flow cytometric analysis was performed to estimate average cellular DNA contents of parental species and hybrid progeny. Whole blood was collected from the caudal veins of 18 individuals per genotype. Blood cells were fixed in cold 70% ethanol and then stained with propidium iodide using a Kinesis 40 kit (Bio-Rad Mississauga, ON, Canada), following the manufacturer's protocol. The stained cell suspension was analysed using a WinBryte HS flowcytometer (Bio-Rad). To estimate average cellular DNA content, human white blood cells (WBC, 7.0 pg $cell^{-1}$) were used as an internal control.

Microsatellite analysis

To confirm the karyogamic status of hybrid progeny, microsatellite analysis was performed using four microsatellite markers that have been developed for winter flounder (D. Cook, in preparation). DNA was isolated from the fin tissues of parental species and 37 hybrids by digesting them in 50 mM Tris-HCl, 100 mM EDTA, pH 8.0, 1% SDS and 100 $\mu g\,mL^{-1}$ of proteinase K at 50 °C for 12 h, followed by one organic extraction with phenol and one with phenol:chloroform (1:1 = v/v). DNA was precipitated using ethanol, washed with 70% ethanol and resuspended in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0); 100 ng of genomic DNA were used as a template for each amplification reaction. The reaction mixture consisted of 0.2 pmol of each primer (Table 1), one of which was end-labelled with a fluorescent tag, 0.2 µM dNTP mix, 10 mM Tris-HCl (pH 9.0), 50 mM of KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, and 2.5 U of *Taq* DNA polymerase (Perkin Elmer, Woodbridge, ON, Canada). Amplification was performed using a thermal cycler PE9700 (Perkin Elmer) and the following amplification conditions: 2 min at 94 °C; 25 cycles of 1 min at 94 °C, 1 min at 50 °C and 1.5 min at 72 °C. The amplified product was analysed using GENESCAN v. 3.1 with Rox-labelled standards (ranging 35–250 bp) (ABI Prism, Perkin Elmer).

Morphometric analysis

At the age of 6 months, 30 fish of each genotype (yellowtail flounder, winter flounder and hybrid) were subjected to morphometric measurement. Ten parameters were measured to the nearest 0.1 mm: standard length (SL), distance between orbits (DBO), snout length (SNL), post-orbital head length (POHL), head length (HL), distance between anterior of dorsal fin base and posterior pelvic fin base (ADFB–PPFB), length of caudal peduncle (LCP), depth of caudal peduncle (DCP), depth of body (DB) and height of arched lateral line (HAL) (Fig. 1). Body proportions were determined as percentages of the above parameters to standard length (SL).

Table 1 Sequences of primers used to amplify winter flounder microsatellites

Primer	Sequence (5′→3′)	Size (bp)	Dye*
79F2	GTC TCT GGG TTT CTA TTG G	146.7, (189.7)	FAM™
79R	TTC CTC CAA CAG CCT CAG		
21F	TGG TAA CAC ACA ACA TGC	194.8, (202.7)	FAM™
21R	GAA GTG GAA TCA TTT AGA C		
27F	AGT GCA ACA ACA GAT TCC AG	142.5, (161.6)	HEX™
27R	GCA GAA TGA GTG AAA TGT GG		
4F	GTG TGG AGG TCA ATG C	82.5, (96.3)	TET™
4R	GGA GCA TCA TTC ATA CAC		

*Single isomer fluorescein dyes from ABI™ genetic analysis system (Perkin Elmer).

Gonad histology

When the fish were 18 months old, gonads were obtained from each of 12 randomly selected yellowtail, winter and hybrid flounders. Ovary and testes were surgically removed from the fish and fixed in Bouin's fixative. Tissues were processed through to wax, sectioned at a thickness of 6 µm and stained with haematoxylin and eosin.

Statistics

Differences in fertilization, hatching, early survival and morphometrics were assessed by ANOVA (followed by Duncan's multiple range test). Raw data of percentage readings were transformed to arcsine before tests. Difference was considered to be significant at the levels of $P = 0.05, 0.01$ or 0.001.

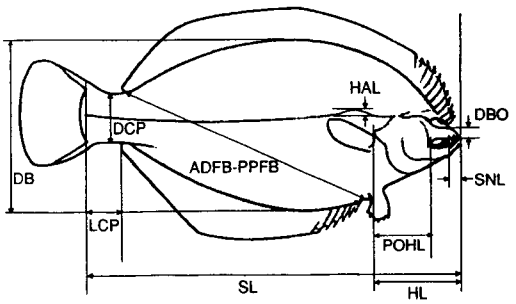

Figure 1 Morphological measurements used to compare interspecific hybrids with their parental species, yellowtail founder, *Pleuronectes ferrugineus* (Storer) and winter flounder, *Pleuronectes americanus* (Walbaum): standard length (SL), distance between orbits (DBO), snout length (SNL), post-orbital head length (POHL), head length (HL), distance between anterior of dorsal fin base and posterior pelvic fin base (ADFB–PPFB), length of caudal peduncle (LCP), depth of caudal peduncle (DCP), depth of body (DB) and height of arched lateral line (HAL).

Results

Fertilization, hatching, abnormality and early survival of hybrids

There was no significant difference in the mean fertilization rate between the two parental pure crosses: 94.7% for yellowtail flounder vs. 96.5% for winter flounder (Table 2). However, the mean hatching success of yellowtail flounder (63.5%) was significantly lower than that of winter flounder (76.5%). The fertilizing ability of winter flounder sperm to yellowtail flounder eggs (81.5%) and hatchability of the resultant embryos (27.0%) were significantly lower than those of their parental pure crosses ($P < 0.001$). Furthermore, higher initial mortality was found in the hybrid genotype: the early survival up to 3 weeks post hatch of the hybrid group (49.8%) was significantly lower than that of yellowtail flounder (73.7%) and winter flounder (86.7%) ($P < 0.01$).

Chromosome number and karyotype of hybrids

Modal chromosome numbers of yellowtail and winter flounders were the same ($2n = 48$), consisting of all acrocentrics (Fig. 2a–c). However, winter flounder had satellites on a pair of relatively large acrocentric chromosomes (Fig. 2c), which were not seen in yellowtail flounder. As expected, the induced hybrids also had also $2n = 48$ acrocentric chromosomes. Hybrid fish had only one acrocentric chromosome containing a satellite, derived from the paternal species, winter flounder (Fig. 2b).

Table 2 Fertilization, hatching and early survival up to 3 weeks post hatch of interspecific hybrids and their parental species, based on three replicate examinations

Genotype (%)	Fertilization (%)	Hatching (%)	Early survival
YT × WT	81.5 ± 2.2^{b}	27.0 ± 2.4^{c}	$49.8 \pm 3.6^{b,c}$
WT	96.5 ± 3.9^{a}	76.5 ± 3.3^{a}	86.7 ± 2.8^{a}

Abbreviations: YT, yellowtail flounder, *Pleuronectes ferrugineus* (Storer); WT, winter flounder, *Pleuronectes americanus* Walbaum); YT × WT, hybrid between yellowtail flounder female and winter flounder male. Early survival rates were determined as percentages of hatched larvae up to 3 weeks post hatch. Means indicated with different letters within a column are significantly different based on ANOVA (followed by Duncan's multiple range test). Percentage data were arcsine transformed before analysis. Means indicated with 'a' are significantly higher than those with 'b' at $P < 0.01$. Means indicated with 'b' are significantly higher than those with 'b, c' ($P < 0.01$) and 'c' ($P < 0.001$).

Average cellular DNA content of hybrids

Representative histograms of flow cytometric analyses used to examine the average cellular DNA contents of parental species and hybrid fish are shown in Fig. 3. The maternal species, yellowtail flounder had an average cellular DNA content of 2.22 ± 0.08 pg cell^{-1}, whereas the paternal species, winter flounder contained 1.78 ± 0.06 pg cell^{-1}. No difference in DNA content was found between sexes within each species. Hybrid flounder possessed

Figure 2 Metaphase chromosome spreads from yellowtail flounder (a), hybrid between yellowtail and winter flounders (b) and winter flounder (c). Arrows indicate the satellites on the chromosomes.

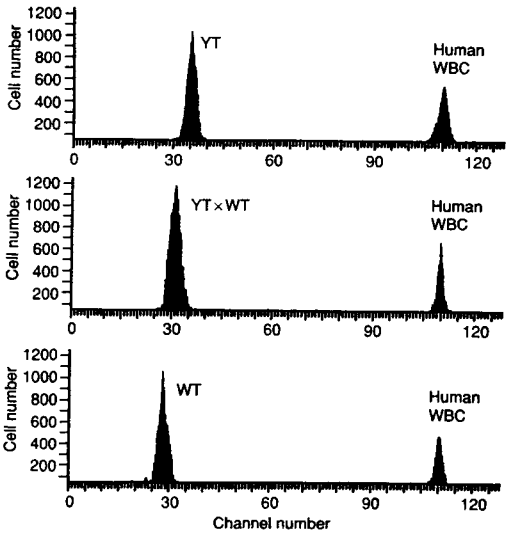

Figure 3 Representative histograms showing the average cellular DNA contents of yellowtail flounder (YT), hybrid between yellowtail and winter flounders (YT × WT) and winter flounder (WT), based on flow cytometry. Peaks from an internal control using human white blood cells (WBC) are also shown.

an intermediate cellular DNA content of 2.02 ± 0.06 pg cell^{-1}.

Microsatellites of hybrids

Microsatellite amplification confirmed that the induced hybrids retained a haploid set of chromosomes from each parental species. Three of the four primer sets (21, 27 and 79F2) developed for winter flounder produced two alleles from winter flounder DNA, whereas one set (primer 4) produced one allele and a null allele. Primer set 4 produced two alleles for yellowtail flounder, whereas primer set 79F2 produced one allele and a null allele (Fig. 4). Primer sets 21 and 27 did not amplify microsatellites in yellowtail flounder. Microsatellite amplification of 42 hybrid fish revealed that most contained one marker from the winter flounder male and one from the yellowtail flounder female, except in the case of primer sets 21 and 27, which failed to give a product for the yellowtail female parent genotype and could only be scored for the male parental genotype (Table 3). One hybrid gave no detectable products with any primer set and five additional hybrids gave no detectable products with three of the four primer sets (failed reactions; Table 3). In addition, six hybrids yielded no detectable products with primer set 27 only. These could result from failed reactions or from hybrids that contained only the female genotype (as primer set 27 amplified no female alleles). Seven samples yielded products for both female alleles with primer set 4, whereas two samples yielded products for both male alleles with primer set 79F2, one of which also yielded products for both male alleles with primer set 21. However, no fish sample that produced both alleles from a single parent with one primer set showed the same phenomenon with the other primer sets. Excluding failed and potentially failed reactions, the genotype expected for hybrids was obtained 83%, 85%, 100% and 94% of the time for primer sets 4, 21, 27 and 79F2 respectively.

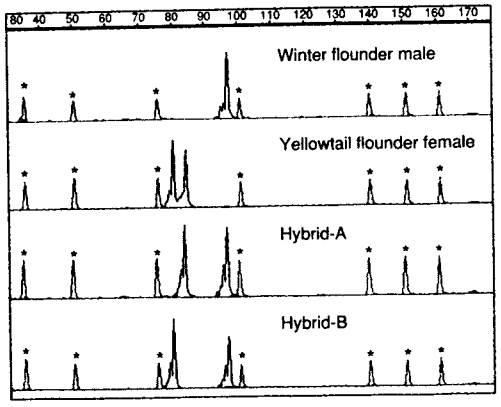

Figure 4 Representative chromatogram microsatellites obtained using primer 4 with DNA from male winter flounder (allele size = 96.3 bp), female yellowtail flounder (79.5 and 83.5 bp) and hybrids between yellowtail and winter flounders (83.5 and 96.3 bp in Hybrid-A; 79.5 and 96.3 bp in Hybrid-B). Note the biparental contribution in microsatellite alleles of hybrid progeny. Asterisks indicate Rox-labelled standard markers (35, 50, 75, 100, 139, 150 and 160 bp).

Table 3 Summary of microsatellite analysis of hybrid progeny between female yellowtail flounder and male winter flounder using four different primer sets

	Primer 4	Primer 21	Primer 27	Primer 79
Female:male (1:1)	34	35	29	32
Female:male (2:0)	7	0	0	0
Female:male (0:2)	0	1	0	2
Failed reactions	1	1	1	6
No products*	0	5	12	2
Total	42	42	42	42

*Lack of products could result from offspring that carried both female yellowtail flounder alleles (which did not amplify with primers 21 and 27) or from failed reactions.

Body proportions of hybrids

Morphometric analyses showed that the interspecific hybrids generally exhibited values intermediate between those of the two parental species (Table 4). Out of nine proportions relative to standard length (SL) examined, there was no difference in one proportion (POHL/SL) among genotypes (yellowtail flounder, winter flounder and hybrid). Yellowtail flounder had significantly higher scores than winter flounder in four parameters (HL/SL, HAL/SL, SNL/SL and ADFB-PPFB/SL). On the other hand, winter flounder had higher values than yellowtail flounder in three proportions (DBO/SL, DCP/SL and LCP/SL) ($P < 0.001$). Generally, hybrid fish displayed intermediate scores that fell between those of the two parental species. Interestingly, hybrid fish showed a higher proportion of body depth to standard length (DB/SL) than both of parental species (Table 4).

Table 4 Body proportions (mean ± SD) of yellowtail flounder, winter flounder and its hybrids ($n = 30$)

Ratio (%)	Yellowtail flounder	Hybrid	Winter flounder
POHL/SL	$15.5 \pm 0.7^{a*}$	$15.5 \pm 0.4^{a*}$	$15.0 \pm 0.6^{a*}$
HL/SL	30.0 ± 1.1^{b}	$26.9 \pm 0.8^{a*}$	$27.1 \pm 0.8^{a*}$
SNL/SL	6.9 ± 0.5^{b}	$6.0 \pm 0.5^{a*}$	$5.9 \pm 0.4^{a*}$
HAL/SL	5.0 ± 0.5^{c}	3.7 ± 0.4^{b}	1.9 ± 0.3^{a}
ADFB-PPFB/SL	$88.9 \pm 1.2^{b*}$	$89.1 \pm 1.0^{b*}$	84.6 ± 1.6^{a}
DBO/SL	0.3 ± 0.1^{a}	0.6 ± 0.1^{b}	1.1 ± 0.2^{c}
DCP/SL	11.1 ± 0.7^{a}	12.6 ± 0.4^{b}	16.8 ± 3.6^{c}
LCP/SL	3.6 ± 0.6^{a}	4.7 ± 0.2^{b}	7.3 ± 1.1^{c}
DB/SL	46.0 ± 1.1^{b}	49.3 ± 0.9^{c}	43.1 ± 3.5^{a}

*Not significantly different at $P < 0.05$. For abbreviations, refer to Fig. 1. Means indicated with different letters within a row are significantly different based on ANOVA ($P < 0.001$).

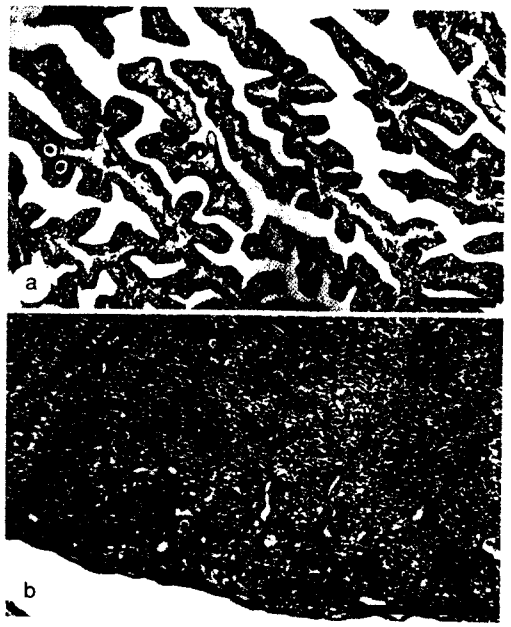

Figure 5 Histological sections of ovary (a) and testis (b) from interspecific hybrid between yellowtail flounder female and winter flounder male. Note the abundance of mesenchymal tissues with oogonia and early oocytes within the ovary and the absence of spermatids or spermatozoa within the testis. Scale bars are 250 μm.

Gonad development of hybrids

Winter flounder and yellowtail flounder had well developed gonads in both sexes at the age of 18 months. Testes from these species had spermatocytes and spermatids, whereas ovaries contained oocytes in various developmental stages: many well-developed eggs were also found in winter flounder and yellowtail flounder females (data not shown). Hybrid individuals of same age displayed retarded gonad development, with the sizes of the ovaries and testes noticeably smaller than those of the parental species. Ovaries from female hybrids contained an abundance of mesenchymal tissues with oogonia and early oocytes (Fig. 5a). These oocytes were significantly smaller in size and fewer in number, when compared with those from yellowtail and winter flounders of the same age. Testes of hybrids contained underdeveloped spermatocytes and there was no clear evidence of successful maturation to spermatids or spermatozoa (Fig. 5b).

Discussion

Induced hybrids, the products of artificial insemination of the eggs of one species by sperm of a genetically dissimilar species, may exhibit the combined geno- and phenotypes of the two parental species (Chevassus 1983). More importantly, in light of the objective of this study, hybrid fishes may display sterility owing to genetic incompatibility of the two haploid chromosome sets originating from different species (Tave 1993; Hulata 1995). To take advantage of this genetic phenomenon, we aimed to produce a hybrid between yellowtail and winter flounders as a means of reproductive containment of a potential exotic species for use in Korean or Asian aquaculture.

In the present study, the insemination of yellowtail flounder eggs with winter flounder sperm produced viable hybrid progeny, although the production efficiency (based on fertilization, hatching and early survival) was significantly lower in the hybrid genotype. However, despite high initial mortality (up to 3 weeks post hatch), there was no further loss in the hybrid group and the survival of hybrids was soon stabilized (data not shown). Although we do not fully understand the reason for the observed early mortality of the hybrids, decreased early survival in hybrid fish has been reported in previous studies (see review, Chevassus 1983; Tave 1993). Further experimentation is needed to examine cumulative mortality through fertilization to ontogenesis of hybrid fry. Also it would be valuable to attempt reciprocal crossbreeding with winter flounder females and yellowtail flounder males as significantly improved results were often obtained in a hybrid cross relative to the

reciprocal cross in Japanese flounder and spotted halibut, *Verasper variegatus* (Temminck & Schlegel) (Kim, Bang, Kim, Nam & Kim 1996).

Overall cytogenetic traits of the hybrids were intermediate between the two parental species as determined by karyotype and DNA content. Most flounder species have been reported to possess a primitive form of karyotype ($2n=48$ acrocentrics; Ohno 1974), which consequently makes it difficult to distinguish the parental genomes in the hybrid flounders using chromosome counts and karyotype analysis (Hoornbeek, MacPhee, Moroz & Seidel 1984). However, fortunately we found satellites on a pair of acrocentric chromosomes in winter flounder metaphase spreads, which were not seen in yellowtail flounder. This allowed us to identify the hybrid status by examining a satellite on an acrocentric chromosome in induced hybrid fish, even though all the genotypes in the present study had the same modal chromosome number ($2n=48$). Based on flow cytometry, the two parental species were found to have different genome sizes. Hybrid flounder had an intermediate average cellular DNA content of 2.0 pg cell^{-1} when compared with the maternal yellowtail flounder (2.2 pg cell^{-1}) and paternal winter flounder (1.8 pg cell^{-1}). The present cytogenetic data are strongly supportive for karyogamic status of the hybrid fish: i.e. the progeny developed from yellowtail flounder eggs inseminated with winter flounder sperm should be true interspecific hybrids rather than spontaneous gynogenetic or androgenetic diploids. Furthermore, the parental genomes were distinguishable in the hybrid using microsatellite amplification.

Four different microsatellite loci confirmed that most of the hybrid genomes consisted of two haploid sets, one originating from each parental species. Seven samples yielded two alleles from only the female parent with primer set 4, but none of these samples exhibited the same pattern for the other primer sets. Similarly, for the samples that yielded two alleles from the male parent with primer sets 21 and 79F2, normal genotypes were found with other primer sets. Unfortunately we have not yet found any clear explanation for this unexpected finding, the presence of two alleles from either the maternal or paternal genomes in several of the hybrids. The only one possible but undefined hypothesis is that this phenomenon might be related with the loss of specific alleles during the artificial hybridization process. However, the possible human error also could not be completely ruled out. Several samples did not amplify detectable products with primer sets 21 and 27 (Table 3). As it was impossible to score for the female alleles with these primer sets, it is difficult to know whether these resulted from failed reactions or elimination of the male alleles at some point during the initial stages of oogenesis. As all of the reactions except one worked correctly with primer set 4, the failure to amplify microsatellites was unlikely to have been low DNA yield or contaminating substances in the DNA sample that prevented amplification. However, it is possible that primer set 4 was more robust than the other sets and able to overcome contaminants in the reaction.

Induced hybrids displayed intermediate morphometrics between maternal and paternal species as has been reported for other fish species (Chevassus 1983). However, there was a slight trend towards the hybrids being more similar to the maternal rather than the paternal species in several parameters including LCP/SL, DCP/SL, DBO/SL and ADFB-PPFB/SL based on *t*-values from separate *t*-tests. Maternal predominance in hybrids has also been reported in hybrids between *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton) (Bhowmick, Jana, Gupta, Kowtal & Rout 1981) and *Poecilia shenops* (Cuvier & Valenciennes) and *Poecilia velifera* (Regan) (George & Pandian 1997). However, in hybrids between channel catfish, *Ictalurus punctatus* (Rafinesque) and blue catfish *Ictalurus furcatus* (Lesueur), congeneric diploid hybrids were more similar to paternal rather than maternal parents (Dunham, Smitherman, Brooks, Benchakan & Chappell 1982).

The hybrid flounder produced in this study are probably sterile, as evidenced by retarded and abnormal gonad development. This is not surprising, as sterility of interspecific hybrids due to genetic incompatibility during meiosis has been reported in other species (for a review, see Chevassus 1983; Tave 1993). Gonadal development followed by regression without maturation has been reported for two-year-old hybrids of smooth *Liopsetta putnami* (Gill) and winter flounders (Hoornbeek *et al.* 1984).

Further study is required to determine the effect of reciprocal crosses (i.e. winter flounder female × yellowtail flounder male) on survival, growth and reproduction in hybrid flounders, because the embryos resulted from the present hybrid cross suffered from high mortality during early ontogenesis. To ensure sterility of the hybrids, rigorous breeding experiments using hormonal treatments may also be needed. Alternatively, crossing of the hybrids themselves could be attempted as a means of

determining reproductive sterility. Before these hybrid flounders can be used in commercial aquaculture in Korea a variety of question about their production characteristics (growth rate, feed conversion efficiency and flesh quality, etc.) need to be answered. Extensive studies are required to examine if these production traits of the hybrid should be sufficiently good to make it a worthwhile proposition for aquaculture compared with indigenous widely used species. Their susceptibility to virus and other pathogens present in Korean waters will also need to be assessed.

Acknowledgments

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References

Beardmore J.A., Mair G.C. & Lewis R.I. (1997) Biodiversity in aquatic systems in relation to aquaculture. *Aquaculture Research* **28**, 829–839.

Bhowmick R.M., Jana R.K., Gupta S.D., Kowtal G.V. & Rout M. (1981) Studies on some aspects of biology and morphometry of the interspecific hybrid, *Catla catla* (Hamilton) × *Labeo rohita* (Hamilton) produced by hypophysation. *Aquaculture* **23**, 367–371.

Brown J., Helm M. & Moir J. (1995) New candidate species for aquaculture. In: *Cold Water Aquaculture in Atlantic Canada*, 2nd edn. (ed. by A.D. Boghen), pp. 341–362. Tribune Press, Sackville, New Brunswick.

Chevassus B. (1983) Hybridization in fish. *Aquaculture* **33**, 245–262.

Dunham R.A., Smitherman R.O., Brooks M.J., Benchakan M. & Chappell J.A. (1982) Paternal predominance in reciprocal channel-blue hybrid catfish. *Aquaculture* **29**, 389–396.

George T. & Pandian T.J. (1997) Interspecific hybridization in Poeciliids. *Indian Journal of Experimental Biology* **35**, 628–637.

Gross M.R. (1998) One species with two biologies: Atlantic salmon (*Salmo salar*) in the wild and in aquaculture. *Canadian Journal of Fisheries and Aquatic Science* **55**, 131–144.

Hoornbeek F.K., MacPhee G.K., Moroz L. & Seidel G. (1984) Experimental hybridization between the smooth flounder (*Liopsetta putnami*) and winter flounder (*Pseudopleuronectes americanus*). *Genetics* **107**, S84.

Hulata G. (1995) A review of genetic improvement of the common carp (*Cyprinus carpio* L.) and other cyprinids by crossbreeding, hybridization and selection. *Aquaculture* **129**, 143–157.

Kim D.S., Jo J.-Y. & Lee T.-Y. (1994) Induction of triploidy in mud loach, *Misgurnus mizolepis* and its effect on gonad development and growth. *Aquaculture* **120**, 263–270.

Kim D.S., Nam Y.K. & Park I.-S. (1995) Survival and karyological analysis of reciprocal diploid and triploid hybrids between mud loach (*Misgurnus mizolepis*) and cyprinid loach (*Misgurnus anguillicaudatus*). *Aquaculture* **135**, 257–265.

Kim K.-K., Bang I.C., Kim Y., Nam Y.K. & Kim D.S. (1996) Early survival and chromosomes of intergeneric hybrids between Japanese flounder *Paralichthys olivaceus* and spotted halibut *Verasper variegatus*. *Fisheries Science* **62**, 490–491.

Litvak M.K. (1999) The development of winter flounder (*Pleuronectes americanus*) for aquaculture in Atlantic Canada: current status and future prospects. *Aquaculture* **176**, 55–64.

Ohno S. (1974) Prochordata, cyclostoma and pisces. In: *Animal Cytogenetics* (ed. by B. John), **Vol. 4**, Chordata 1, pp. 1–91. Gebr. Borntraeger, Berlin.

Saegrov H., Hindar K., Kalas S. & Lura H. (1997) Escaped farmed Atlantic salmon replace the original salmon stock in the River Vosso, Western Norway. *ICES Journal of Marine Science* **54**, 1166–1172.

Tave D. (1993) *Genetics for Fish Hatchery Managers*, 2nd edn. Van Nostrand Reinhold, New York.

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