

Segregation distortion at a microsatellite marker in the olive flounder, *Paralichthys olivaceus*

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Abstract: In the course of quantitative trait loci (QTL) analysis of the back cross (BC₁) families of olive flounder (*Paralichthys olivaceus*), we observed significant segregation distortion at a microsatellite marker, Poli9-58TUF in two crosses of informative progenies. The family 1 of the random BC₁ progenies derived from a cross between a F₁ male genotype (A/B) and a F₁'s female parent genotype (A/C) and the family 2 (A/C x A/C) displayed a strong bias in the locus from the Mendelian inheritance by the elimination of homozygous A/A genotype. The deleterious roles of the AA genotypes are suggested during the metamorphosis and it implies that the parents of these families carried a recessive gene or genes hampering development at an early stage because the offspring of the double heterozygote parents show the reduction in frequency or elimination of one homozygous class, which is an evidence for linkage between the genetic marker and gene(s) with recessive deleterious alleles. This data support a hypothesis that the region contains a recessive lethal gene or genes.

Key words: Segregation distortion, Microsatellites, *Paralichthys olivaceus*, Lethal gene
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Introduction

Skewed segregation ratios of genetic loci are often observed in the progeny of inter- and intra-specific hybrids and the phenomenon are resulted from distorted inheritance of alleles caused by competition among gametes or from abortion of gamete or zygotes for preferential fertilization (Lyttle, 1991).

Segregation distortion of genetic markers has been used to identify meiotic drive (Ruvinsky, 1995), genomic imprinting (Solter, 1988) or loci with deleterious effects on embryonic development (Lie *et al.*, 1994; Renard *et al.*, 1994; Weichenhan *et al.*, 1996; Stizinger *et al.*, 1999). McGoldrick and Hedgecock (1997) reported that several characteristics of distorted segregation ratios are that, departures from classical Mendelian ratios occur in crosses of individuals collected from the wild but is exacerbated in families known to be inbred or in samples taken from small hatchery stocks as a result of genetic load after inbreeding in the inbred families of the Pacific oyster. Deviations from the expected Mendelian segregation ratios are not uncommon, having been reported in many plants (Xu *et al.*, 1997; Faris *et al.*, 1998) and animals (Yasue *et al.*, 1999; Mikawa *et al.*, 2005) as well as in some of aquaculture species (McGoldrick and Hedgecock, 1997; Launey and Hedgecock, 2001; Palti *et al.*, 2002).

In this study, we observed significant segregation distortion at a microsatellite marker in the course of QTL analysis of olive flounder families. Here, in order to produce the reference families, back cross (BC) of F₁ male sires to their female parents produced the BC generation and gene segregation was recorded both in BC

and in control families to reveal the segregation pattern of the alleles at different developmental stages.

Materials and Methods

Production of reference families and collection of samples: The parental hatchery brood stock of olive flounder *paralichthys olivaceus* which are kept at Genetics and Breeding Research Center of NFRDI in Korea were individually tagged with a passive integrated transponder (PIT) tag (Biomark, Boise, Idaho, USA). The hatchery brood stocks were artificially crossed in 2004 and two F₁ males from each pair mating were randomly chosen and two backcrossed F₂ families were made from crosses between the F₁ males and the F₁'s female mothers (Fig. 1). The crosses between individuals are listed in Table 1. Genotyping of one hundred individuals within each family was done twice at 25 days (during the metamorphosis at WT 19-20°C) and at the juvenile stage, 105 days after fertilization.

Microsatellite analysis: DNA was extracted from fin samples using TNES-urea buffer (6 M urea, 10 mM Tris-HCl (pH7.5), 125 mM NaCl, 10 mM EDTA, 1% SDS) and proteinase K treatment followed by standard phenol extraction method (Asahida *et al.*, 1996). PCR reactions were performed in 10 µl reaction volumes containing 50 ng of genomic DNA, 10 mM Tris-HCl (pH 8.8), 0.1% Triton X-100, 5 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 5 pmol of each primer and 0.5 units Taq DNA polymerases (Promega). Amplification was carried out using a PTC 200 MJ-Research thermocycler DNA engine in the following conditions: an initial denaturation at 95°C for 15 min followed by 35 cycles of 20 s at 94°C, 40 s at primer-specific annealing temperature between 58°C and 62°C, 1 min at 72°C and



a final extension period of 10 min at 72°C. For fluorescent detection of the PCR products, the forward primer in each pair was labeled with 6-FAM, NED or HEX dyes. Polymorphisms at the microsatellite markers were screened using an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA) and alleles were designated according to the PCR product size relative to a molecular size marker (GENESCAN 400HD ROX, PE Applied Biosystems). Genotypes were scored using the GENESCAN (ver. 3.7) and the GENOTYPER (ver. 3.7) software.

Results and Discussion

In the course of QTL analysis of Olive flounder families, we observed the segregation distortion of allele as Mendelian manner at the microsatellite DNA marker Poli9-58TUF (GenBank accession no. AB086483 and AB086484) in the grown up stage. In the cases of family 1 and 2, the absence of homozygous (131/131) genotype leading to a significant distortion from the expected Mendelian was detected at 105 days after fertilization (Table 2). Samples were genotyped with Poli9-58TUF marker in order to see whether homozygote (131 allele) individuals were existing in the group that was formed from nature and farmed populations. A total of 25 alleles at this locus were identified in 132 individuals and the size range of the PCR products was from 115bp to 181bp. Allele frequency distributions are shown in Fig. 2. Two types of homozygotes at this locus were detected, 121/121 and 135/135 respectively with proportion of 19.7% and 2.3%. Furthermore homozygous genotypes of 141/141 (family 2 and 3) and 121/121 (family 4) were observed according to the

Table - 1: Developmental stages and the sample size used for the individual genotyping analysis of olive flounder

Family ^a	Stage (d.a.f.) ^b	Sample size
1 (CA022 × CC156)	23	100
	105	100
2 (CJ053 × CC156)	23	100
	105	100
3 (CJ053 × CC106)	105	100
4 (CA022 × CC106)	105	100

^aThe parents of each family in name tags (dam × sire)

^bDevelopmental stages were measured in days after fertilization (d.a.f.)

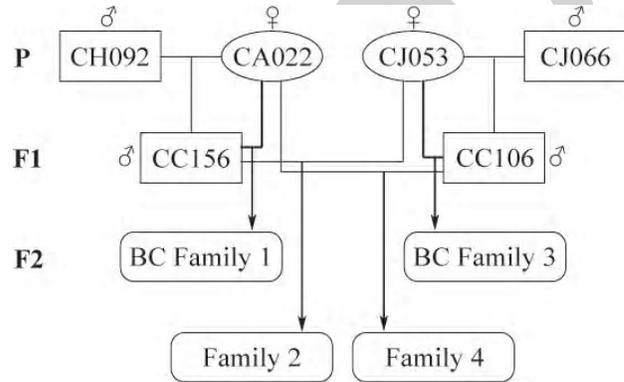


Fig. 1: Breeding scheme used to produce the reference families of olive flounder, *Paralichthys olivaceus*

Table - 2: Genotypes of the parents and genotypic segregation in their progeny for the Poli9-58TUF locus

Fam.#	Parents		Stage (d.a.f.)	Progeny						Expected ratio
	Dam	Sire		AA	AB	AC	BB	BC	CC	
1	131/121(AB)	131/141(AC)	25	48	10	23	-	19	-	1:1:1:1
			105	0	30	13	-	57	-	1:1:1:1
2	131/141(AC)	131/141(AC)	25	53	-	29	-	-	18	1:2:1
			105	0	-	79	-	-	21	1:2:1
3	131/141(AC)	121/141(BC)	105	-	30	13	-	25	27	1:1:1:1
4	131/121(AB)	121/141(BC)	105	-0	25	18	30	27	-	1:1:1:1

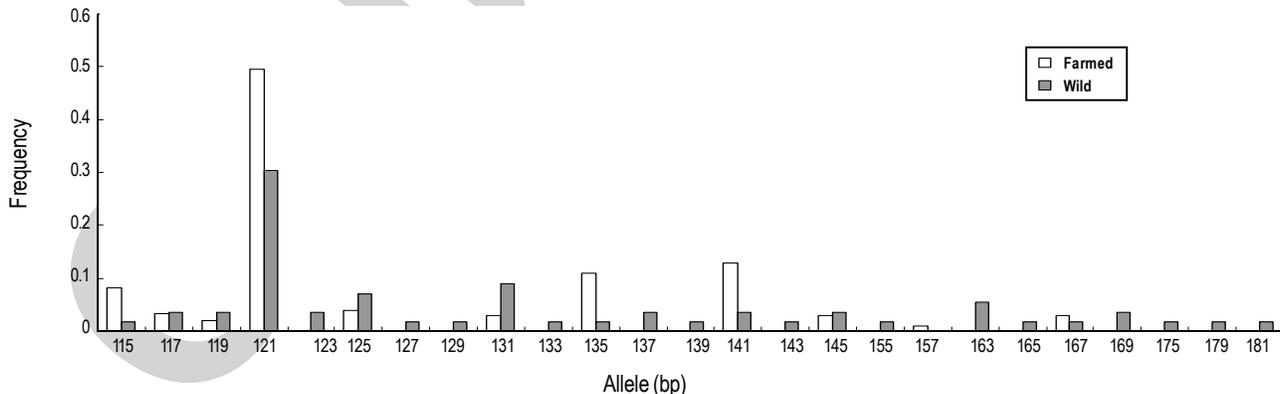


Fig. 2: Allele frequency distributions of the farmed and wild olive flounder populations at Poli9-58TUF locus



Mendelian ratio. However, 131/131 homozygote was not only in the olive flounder random brood populations but also in the family lines (family 1 and 2) at the grown up stage.

On the other hand we observed the excess of 131/131 homozygote in family 1 and 2 at the 25 days after fertilization. It means that the progenies with 131/131 genotype still survived until this stage.

In general, the metamorphosis of the olive flounder is around at days 23 to 30 after fertilization at the optimum water temperature around 20°C. The fast developed progeny settle down on the bottom of tanks and meanwhile the lagging individuals were still swimming in the water surface. The swimming fish were randomly captured and compared with the majority of bottom settled fish at 25 days after fertilization. The AA homozygote frequency was much high in the slow developing fish group. This result suggests that the deleterious process may occur between 25 and 105 days after fertilization and Pol9-58TUF marker is closely linked with the gene(s) responsible for the process. This distortion could be related to the developmental failure of embryos containing the alleles. Similar findings were reported for a swine mapping resource family (Uenishi *et al.*, 2000; Yasue, 2000). These authors determined that termination of fetus development was the cause for segregation distortion of DNA markers. Palti *et al.* (2002) found that three microsatellite loci from the fourth generation of the meiogynogenetic line of tilapia were found to be linked to genes with deleterious alleles at hatching stage.

The pattern of early mortality associated with identical-by-descent (IBD) marker homozygote in plants supports the hypothesis of McGoldrick and Hedgecock (1997) that inbreeding depression in the early life stages is caused mainly by lethal rather than mildly deleterious mutations. It also seem to be the case in this study that most of the mortality in Oliver flounders occurs in the phase of late larval stages or around the metamorphosis. When both parents are heterozygous at a locus, the reduction in frequency or elimination of one homozygous class in the progeny is an evidence for linkage between the genetic marker and gene(s) with recessive deleterious alleles (Palti *et al.*, 2002). Taken together, our observations indicate that a gene or genes malfunctioning at an early stage of fetal development terminates further development.

The follow-up study will determine the time of fetal development at which the lethal gene(s) is activated. Knowledge of the segregation distortion loci will aid breeders in designing appropriate crossing schemes and a breeder may be able to maximize transmission of desired alleles or to preferentially exclude deleterious alleles.

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