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Parental contribution and growth hormone gene polymorphism associated with growth phenotypes of red sea bream *Pagrus major* in mass production: A case study

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ABSTRACT

Red sea bream is one of the most important aquaculture fish species in Japan. To improve the productivity of this fish during seed production, improved growth traits and reduced size variation are needed. In this study, we assessed parental contribution of fast- and slow-growing individuals observed in two different rearing phases in a mass production lot: (1) 50 dph reared in a tank and (2) 200 dph reared in a net cage. We also assessed GH gene (*pmaGH*) polymorphisms based on a previously developed minisatellite DNA marker. Specific broodstock individuals were significantly associated with fast- or slow-growing individuals at 50 dph and 200 dph. Significant differences in *pmaGH* minisatellite allele frequencies were observed between fast- and slow-growing groups at 50 dph in the frequency of two alleles (*pmaGH*-740 and *pmaGH*-900, respectively). Combining the results of DNA parentage analysis and *pmaGH* minisatellite allele analysis, one dam and two sires, possessing *pmaGH*-740, were significantly associated with the slow-growing groups. These results suggest that the minisatellite marker of *pmaGH* could be a useful tool for growth selection of this fish species.

1. Introduction

Red sea bream (*Pagrus major*) is one of the most cultured fish species in Japan because of palatability and high economic value. Nowadays, around 80,000 tons of this species is cultured per year, which is approximately 5-fold the size from catch (Miyashita and Seoka, 2005). This species also has the longest history of domestication of marine finfish species in the world (Miyashita and Seoka, 2005), and a strain was successfully developed showing faster growth than non-selected fish over 25 years of the intensive breeding program conducted by individually selecting phenotypes for growth performance (Murata et al., 1996). We previously identified genetic divergence of domesticated stocks of red sea bream based on microsatellite DNA markers, and aquaculture stocks of this species still have enough genetic diversity as a gene pool (Sawayama and Takagi, 2014a). This large genetic diversity is possibly a cause of fluctuation in seed quality in each production lot, and broodstock and family selections are needed for stable production of robust seedlings.

Extremely fast- and slow-growing seedlings of red sea bream are observed during artificial seed production. Both phenotypes are observed in every production lot, but the observation ratios of these phenotypes varied. The possibility of different observation rates of these seedlings in each production is suggested as differences in environmental factors: water temperature, nutrients, and labor skills. However, the broodstock of this fish species still has large genetic variability (Sawayama and Takagi, 2014a), and genetic factors are suggested as one cause of size variants in this species. Recently, DNA parentage analysis using microsatellite DNA markers has become a common methodology for family selection of mass spawning finfish in the aquaculture industry (Garber and Sullivan, 2006; Sawayama and Takagi, 2014b), and this method has been used to identify broodstock, which is associated with offspring having robust or poor economic traits (Peruzzi et al., 2006; Borrell et al., 2011; Sawayama and Takagi, 2011, 2012; Loughnan et al., 2013). Hence, broodstock selection using DNA parentage analysis is one possible solution to improve growth traits.

We previously isolated the growth hormone gene of red sea bream (*pmaGH*) and developed a highly polymorphic tandem repeat marker locating intron 3 of *pmaGH* (Sawayama and Takagi, 2015). Growth hormone is the main regulator of postnatal somatic growth and stimulates anabolic processes such as cell division,

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skeletal growth, and protein synthesis (Goodman, 1993). Several studies have reported the *GH* gene as a candidate for enhancing several production traits in livestocks (Hoj et al., 1993; Knorr et al., 1997; Kuhnlein et al., 1997; Dybus, 2002; Tambasco et al., 2003) and also aquaculture fish species (De-Santis and Jerry, 2007). Therefore, *GH* gene polymorphisms are thought to be useful for marker-assisted selection (MAS) to improve growth performance in red sea bream.

In the present study, we conducted DNA parentage analysis to identify broodstock associated with extremely fast- and slow-growing seedlings at two different rearing phases: (1) 50 days-post-hatch (dph) cultured in a tank, and (2) 200 dph cultured in a net cage after transportation to the open sea. The variability of the *pmaGH* polymorphisms was also analyzed based on the *pmaGH* -located minisatellite marker, and allele frequency of *pmaGH* between size groups was compared. Finally, we discuss parental contribution and *pmaGH* allele inheritance between size groups.

2. Materials and methods

2.1. Fish

A total of 51 broodstock (19 dams and 32 sires) were used for seed production. The broodstock were allowed to spawn freely in September 2011. Fertilized eggs from two days were collected and introduced to a 50 kL tank at a density of approximately 15,000 eggs/kL. Water temperature was maintained at 20 ± 1.5 °C until 30 dph using a chiller that was installed in the system, and after that no control of water temperature was done (ranged 19.3–23.5 °C). Tanks were supplied with through-flowing seawater, which had been previously filtered by a sand filter and pass through a UV sterilizer. Salinity was approximately 35‰, the oxygen level was maintained above 6 mg/L using liquid oxygen, and photoperiod was controlled at 12 h light and 12 h dark. Water exchange was ranged 30–500%/day. Addition of small-type rotifers, *Brachionus* sp. "Cayman", to the rearing tank was started at 4 dph, and feeding densities were set at 5–10 individuals/mL in accordance with larval growth until 25 dph. From 20 to 35 dph, *Artemia* nauplii were added once to forth in accordance with larval growth. Rotifers and Artemia were enriched with the DHA enrichment material (Bio Chromis, Chlorella Industry Co., Ltd., Tokyo, Japan). Fish were manually fed commercial aquaculture diets (Otohime, Marubeni Nissin Feed Co., Ltd, Tokyo, Japan; Jr., Maruha Nichiro Corporation, Tokyo, Japan) from 30 dph. At 35 dph, approximately 200,000 juveniles were transferred to another 50 kL tank to reduce the rearing density. At 50 dph, these fish were size graded using a screen-type automated live fish grader (Matsusaka Ltd., Osaka, Japan) and fish over 45 mm in total length (approximately top 60%) were moved to net cages off the coast of Iwagi Island (34°15′N, 133°09′E,) in the Seto Inland Sea. 50,000 individual each was introduced into a net cage (10 × 10 × 6 m) and cultured until 200 dph. Fish were manually fed commercial diets (EP, Maruha Nichiro Corporation, Tokyo, Japan) once a day after transportation to the offshore net cage.

For genetic analysis, we first collected 1000 individuals at 50 dph and measured body weight (g) as a growth trait. Then, one hundred individuals each of the top 10% (3.2 ± 0.3 g) as a fast-50 group, the bottom 10% (0.6 ± 0.6 g) as a slow-50 group, and average ±5% (2.0 ± 0.2 g) as a medium-50 group were collected based on body weight (Fig. 1a). Moreover, to compare parental contribution to growth after transportation to the offshore net cage at 200 dph, two groups of this population were sampled: 50 fish with good growth performance (44.5 ± 5.0 g) as a fast-200 group and 50 fish with bad growth performance (12.6 ± 2.9 g) as a slow-200 group (Fig 1b). The fish making up the two groups at 200 dph were identified based on body length as selected by the fish breeder. All specimens were confirmed to be without any malformations based on visual observation. These specimens were stored in 99.5% ethanol or at −20 °C for further genetic analysis.

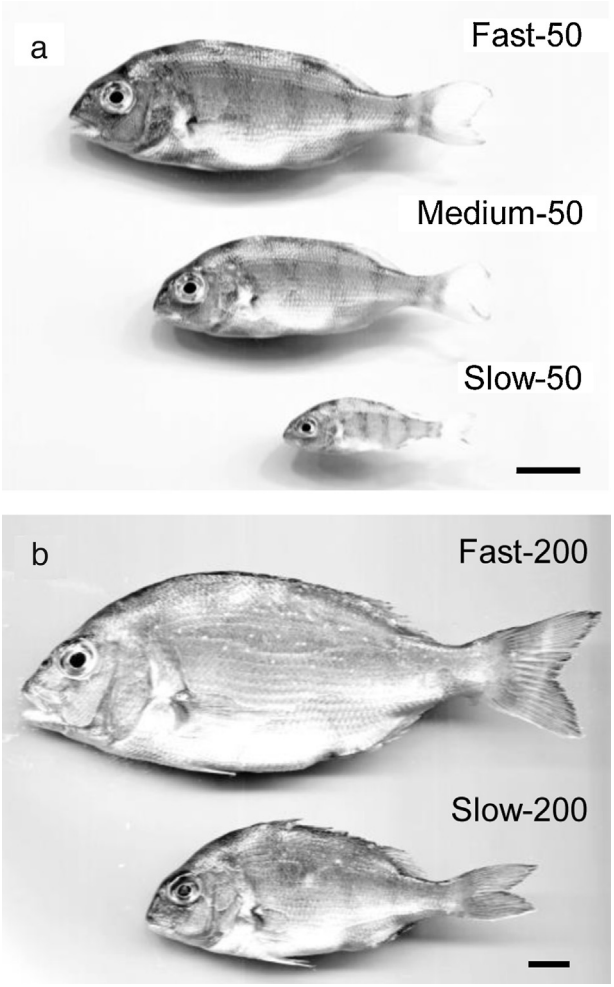

Fig. 1. Photographs of (a) typical fast-, medium-, and slow-growing fish individuals at 50 dph, (b) typical fast- and slow-growing fish individuals at 200 dph. Bars indicate 10 mm.

2.2. DNA extraction and allele detection

Genome DNA was extracted using a High Pure PCR Template Preparation Kit (Roche Diagnosis, Tokyo, Japan) from portions of caudal fin of specimens according to the manufacturer's protocol. The primers of eight polymorphic microsatellite loci, *Pma-2**, *-3**, *-4**, and *-5** (Takagi et al., 1997); *Pma22-9**and *Pma4-32** (Hatanaka et al., 2006); and *Kpm2**and *Kpm22** (Blanco-Gonzales et al., 2012) were used as neutral markers and amplified using polymerase chain reaction (PCR). The general PCR protocol was 50 ng of extracted DNA, 0.1 μM primer, 0.2 μM deoxynucleotide triphosphates (dNTPs), 0.05 μL 99% formamid, and 2.5 units Ex taq polymerase (Takara Bio, Shiga, Japan) with 10 × buffers to a total volumes to 5 μL. The PCR reactions were carried out on a PC816 (Astec, Fukuoka, Japan) using the following profile: 30 cycles of 95 °C for 30 s, annealing temperature for each primer pair for 30 s, and 72 °C for 30 s. The annealing temperature followed a previous study (Sawayama and Takagi 2014a). Forward primers were labeled with fluorescent dyes, and reverse primers were tailed (Applied Biosystems, California, USA). The PCR products were multi-loaded and separated by electrophoresis using an ABI Prism®