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QTL analysis of growth-related traits in kelp grouper *Epinephelus bruneus* and tiger grouper *Epinephelus fuscoguttatus* for selective breeding program

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博士学位論文内容要約 Summary

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論文題目 Title	QTL analysis of growth-related traits in kelp grouper <i>Epinephelus bruneus</i> and tiger grouper <i>Epinephelus fuscoguttatus</i> for selective breeding program 選抜育種プログラムのためのクエおよびアカマダラハタにおける成長に関連するQTL解析		

Since the recent decade, grouper has become a commercial important aquaculture fish in East Asia. This is due to the reasons of its high quality of meat and low quantity of catch from natural waters, and these lead groupers to become high in market demand and price. Many species of grouper have been successfully bred and cultured in Asia. However, the grouper culture still has been encountering many of common problems such as viral nervous necrosis disease (VNN), low survival rate in larval stage and slow growth rate to the marketable size. Kelp grouper and tiger grouper which are commercially important fish in Japan and Thailand, are good examples of these common problems especially their slow growth rate that result in prolonging of farming period and shortage of grouper supply to domestic and international seafood markets.

In order to solve these problems, slow growth rate is selected as a crucial priority for scientist to improve by the breeding program. Among several techniques in fish breeding, a genetic improvement using Marker Assisted Selection (MAS) seems to be currently an effective tool of solving this slow growth due to its high ability of providing high resolution of genetic information for breeding program based on family selection. Thus, a Genetic marker, Genetic linkage map and Quantitative trait loci (QTLs) for MAS are strongly required to meet the achievement of this study.

In 2013, the second generation genetic linkage maps and the first QTLs analysis of growth related traits in the kelp grouper were constructed using a parent and 90 progeny with 470 microsatellite markers. The results showed that the genetic linkage maps of kelp grouper are covering 23 and 24 linkage groups in the female and male maps, with marker intervals of 6.1 and 5.0 cM, respectively. The result of the first QTLs analysis detected the major QTLs with 5 markers on genome and chromosome wide significance in linkage group EBR17F and 19 putative QTLs in 5 different linkage groups. In this study, the QTLs regions related to growth trait in kelp grouper was confirmed for finding out the essential markers that could be able to universally use for the detection of growth related traits genes in the other family of kelp grouper and also in other species of grouper. A number of makers and progenies and referent families were increased for the accuracy of QTLs regions and finding out of the essential markers in kelp grouper. These markers were firstly tested for constructing the linkage maps and QTLs analysis of growth related traits in tiger grouper in order to evaluate the universal property in both species. The results were summarized in three chapters.

Chapter 1: Genetic linkage maps and Quantitative trait loci (QTLs) of growth-related traits in kelp grouper

The - major QTLs regions of kelp grouper were marked using 1,055 microsatellite markers and a high-resolution genetic linkage map was constructed in an F₁ family A. Genome-wide and chromosome-wide significances of the growth-related QTLs (body weight: BW and total length: TL) were analyzed using

non-parametric mapping, Kruskal-Wallis analysis, simple interval mapping (IM) and a permutation test (PT). Two stages of two families were used to confirm the QTLs region. The results showed that about 714 SSR markers were matched which evenly covered the 24 linkage groups and about 509, 512 markers were also identified in the female and male maps. The genome lengths were approximately about 1,475.95 cM and 1,370.39 cM which covered 84.68% and 83.21% of the genome in kelp grouper, with an average interval of 4.1 cM and 4.0 cM, in females and males, respectively. One major QTL related to the body weight and total length was found on linkage group EBR 17F that identified for 1% of the genome-wide significance and accounted for 14.6–18.9% and 14.7–18.5% of the phenotypic variance. Several putative QTLs with 5% chromosome-wide significance were detected on 8 linkage groups. Furthermore, the confirmed results of the regions harboring the major and putative QTLs showed consistent significant experiment-wide significance of 1%, 5% and a chromosome-wide significance of 5%.

Chapter 2: Genetic linkage maps and Quantitative trait loci (QTLs) of growth-related traits in Tiger grouper

Three referent families (Family A, B and C) of the tiger grouper were prepared for the construction of genetic linkage map and QTLs analysis of growth related traits in tiger grouper. A parent and 90 progeny of family A were used to construct genetic linkage map and candidate QTLs regions. The other families (family B and C) were also used to confirm the QTLs region. The amount of 456 microsatellites markers were used for the construction of a linkage map with family A. Twenty-four genetic linkage groups (EFU1-EFU24) were identified. The results showed that female linkage map contained 374 markers that distributed in 24 linkage groups (EBR 1F-EBR 24F). The total genome size of the female map was estimated about 1,770.71 cM and the number of markers per linkage group varied from 7 to 31, with an average of 16. The longest linkage group of the female map was extended to 99.3 cM (EFU 1F). Meanwhile, about 322 markers were distributed in 24 linkage groups of the male map (EFU 1M-EFU 24M). The total genome was estimated about 1,583.37 cM in the male map. The longest linkage group of the male can also be extended to 84.2 cM (EFU 23M). The average number of markers per linkage group was about 13 and varied from 9 to 31. The average interval markers were about 5.66 cM and 5.53 cM, respectively. The relative recombination ratio between female and male in these pairs was about 1.12:1, which indicates the higher recombination rate of the female linkage groups than the male. Two majors QTLs related to the tiger grouper body weight were found on 2 linkage groups (EFU12, EFU21). The first major QTL was found in the linkage EFU12F and consisted of two loci that identified for 1% of genome-wide significance and accounted for 21.8 % of the phenotypic variance. While, the second major QTL was found in linkage groups EFU21F and EFU21M and consisted of two and one loci which identified for 5% of genome-wide significance and accounted for 17.6%, 15.1% of the phenotypic variance respectively. Then, about 9 representative microsatellite markers from linkage groups EFU12F, EFU21F and linkage group EFU21M were used to confirm the major QTLs region with 500 progeny of tiger grouper family A, 270 progeny of family B and 262 progeny of family C. The confirmed results showed a consistent significant experiment-wide significance of 1% of the two major QTL regions in two linkage groups of family A. In addition, one major QTL regions on linkage EFU21F and EFU21M of family B were confirmed and showed a consistent significant experiment-wide of 5% and 1%, respectively. Moreover, the new QTL region in linkage group EFU12M was found with one marker and showed a consistency of the significant experiment-wide

significance of 1%. Finally, the confirmation in family C was conducted and the results showed a consistent significance in only one QTL region on linkage group EFU21F of about 1% experiment-wide significance. In addition, we also analyzed the QTLs of growth related trait with a different stage of family A (from state 1 to stage 11). The results showed that two majors QTLs affecting BW was started from stage 5 until stage 12 on same QTLs regions. There results suggest the possibility of this technique to determine the minimum of the suitable period for detect QTLs of growth related trait of tiger grouper.

Chapter 3: Comparative quantitative trait loci (QTLs) of growth-related traits in kelp grouper and tiger grouper

The result in this study reveals that the growth related trait QTLs of kelp and tiger groupers are presented in the different linkage groups even though almost of the markers are located in the same order and the same linkage group. As the results, we would be able to conclude that the QTLs markers could be applied to evaluate the growth related traits of other family in same species and are specific with each species.