

# Genomics and genetics of flatfishes provide insights into the mechanisms of sex determination and disease resistance

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博士学位論文内容要旨  
Abstract

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論文題目 Title	Genomics and genetics of flatfishes provide insights into the mechanisms of sex determination and disease resistance 異体類のゲノム科学と遺伝学は性決定と耐病性のメカニズムの解明に手掛かりをもたらす		

Flatfishes, members of the order Pleuronectiformes, comprise a biologically interesting and commercially important species, with a unique asymmetric body shape developed as an adaptation to a lifestyle living on the sea bottom. As a commercially important cultured flatfish, half-smooth tongue sole (*Cynoglossus semilaevis*) have a great growth dimorphism that females grow faster and attain larger size than males. Besides the tongue sole possessed a ZZ/ZW genetic sex determination system and the functional sex reversal can be induced by environmental factors. Therefore, the coexistence of GSD and ESD system within the tongue sole, coupled with the availability of its genomic and genetic information, provides an opportunity to comprehensively investigate the mechanism of sex determination and differentiation of this species. Besides, another target flatfish species, Japanese flounder (*Paralichthys olivaceus*) is also one of the most desirable and highly priced marine fish species. With extensive cultivation, however, farming of the Japanese flounder has been confronted with certain problems, including a high mortality rate as well as a decline in growth. To further increase profitability and sustainability while maintaining genetic variability in the cultured stock, it is urgent to carry out both classic selective breeding and molecular marker assisted selection. In this thesis, a multi-level of genomic and genetic analysis focuses on the sex determination and differentiation as well as the disease resistance of flatfishes were carried out. The main results are as follows:

Two bacterial artificial chromosome (BAC) libraries for tongue sole were constructed in the *Bam*HI and *Hind*III sites of the vector pECBAC1. The two libraries contain a total of 55,296 BAC clones with average insert size of approximately 156.4 Kb and correspond to 13.36 haploid genome equivalents. The combined libraries have a greater than 99% probability of containing any single-copy sequence. Screening high-density arrays of the libraries generated 76 and 152 positive clones with average of 15.2 and 30 per probe for sex-related genes and female-specific markers. Five positive BAC clones containing sex-related genes and female-specific markers were sequenced by ABI 3730 DNA analyzer. A full-length BAC sequence with nine genes including *cyp19a1a* gene was further analyzed on the structural characteristics such as repeats, GC content, transposable elements, etc. A comparative analysis on the *cyp19a1a* gene was carried out among medaka, zebrafish, stickleback, *Fugu rubripes* and *Tetraodon*, suggesting that the conservation of *cyp19a1a* gene structure and function, mainly including the same pattern of exons and introns, five conserved functional domains, the common upstream regulatory elements and the high expression in ovarian specificity for all the teleost *cyp19a1a* gene.

The single-base-resolution methylomes for tongue sole were depicted using the whole genome bisulfite

sequencing (WGBS). In total, 171 Gb methylome data were produced, which yielded an average depth of 22× per strand for adult gonads of parental females (P-ZWf), parental pseudomales (P-ZWm), F1 pseudomales (F1-ZWm), and females (F1-ZWf) as well as the normal male (ZZm) as a control. The results show that the methylation patterns of pseudomale testes were similar to those of normal male testes, and all three-testis samples were clearly distinguished from the ovary samples by hierarchical clustering analysis. Only 60 Kb of differentially methylated regions (DMRs) were identified between P-ZWf and F1-ZWf. In contrast, an average of 15 Mb of DMRs was identified between testes and ovaries. Interestingly, up to 86% of the DMRs between P-ZWm and ovaries were maintained in F1-ZWm, indicating that the overall change in methylation status of the genome after sex reversal had been inherited. Besides, 2,986 differentially methylated genes (DMGs) that harbored testis/ovary DMRs on their promoter regions were identified. In total, 16 of 58 (28%) sex-determination-related genes in tongue sole displayed significantly differential methylation patterns between testes and ovaries, which contrasts to 14% (2,986/21,516) over the whole genome, suggest that the sex determination pathway is the target of DNA methylation regulation. Transcriptome analysis revealed that the dosage compensation occurs in a restricted, methylated cytosine enriched Z chromosomal region in pseudomale testes, achieving equal expression level in normal male testes. In contrast, female-specific W chromosomal genes are suppressed in pseudomales by methylation regulation.

A total of 12,712 high-confidence SNPs were successfully high-throughput genotyped and then were assigned to the 24 consensus linkage groups (LGs) based on the restriction-site associated DNA (RAD) sequencing in Japanese flounder. The total length of the genetic linkage map was 3497.29 cM with an average distance of 0.48 cM between loci, thereby representing the dentist genetic map in Japanese flounder. Ten quantitative trait loci (QTLs) forming two main clusters for *vibrio anguillarum* disease were detected. All QTLs with logarithm of odds (LOD) value ranged from 3 to 15.8 can explain the total of phenotypic variance from 1.19% to 8.38%. By synteny analysis of the QTLs regions with genome assembly, twelve immune related genes were identified and among them, 4 genes involved in the function of MHC were strong associated with *V. anguillarum* disease. Furthermore, 246 genome assembly scaffolds with an average size of 21.79 Mb, accounting for 522.995 Mb totally, were anchored onto the LGs, representing 95.8% of the assembled genomic sequences of the Japanese flounder. The mapped assembly scaffolds in Japanese flounder were then used for genome synteny analysis against the zebrafish and medaka, respectively. The flounder and medaka have almost one-to-one synteny, while the flounder and zebrafish have multi-synteny correspondence, indicated that the medaka has a closer relationship with flounder.