ABSTRACTS

SESSION 1: BIOTECHNOLOGY AND FUNCTIONAL GENOMICS

UNDERSTANDING GENETIC RESISTANCE TO VIRAL INFECTION IN ATLANTIC SALMON USING PRIMARY AND IMMORTILISED CELL CULTURE MODELS.

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The Atlantic salmon breeding industry uses family-based and genomic selection for disease resistance. Typically, disease challenges of juvenile full siblings of the selection candidates from each family of the breeding program is required to obtain accurate breeding values. Studying genetic resistance to viral infection using cell culture systems, in particular embryonic primary cell, offers a platform to (i) understand the factors underlying genetic resistance, and (ii) a potential model system to reduce the need for the yearly disease challenge experiments. To this end, we have been using primary and immortalised cell culture models to study genetic resistance to viral disease, using infectious pancreatic necrosis virus as an exemplar case. These experiments have included development of a primary cell platform from embryos from individuals of known resistance genotype to test the relationship between *in vitro* resistance, and expected genetic resistance based on genotypes at a major QTL. Preliminary results suggest that genetic resistance is partially mirrored in the primary cell culture. This opens the possibility of using primary cells to study and identify the functional mechanisms underpinning genetic resistance. Experiments are underway to assess the efficacy of CRISPR-Cas9 targeting of specific candidate genes, and the impact on the cell culture phenotype in immortalised and primary cells. Further we plan to test the efficacy of the primary cell system to screen for genetic resistance to other viral pathogens of importance to salmon aquaculture. If successful, this platform could dramatically reduce the number of animals used for evaluating the within and between family resistance to multiple viral infections in breeding programs. It also has potential as a platform to understand mechanisms of genetic resistance with reduced need for animal disease challenge experiments.

Keywords:
CRISPR-Cas9, Primary cells, Atlantic salmon, Disease resistance

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NEXT GENERATION SEQUENCING AND QUANTIFICATION OF MAJOR HISTOCOMPATIBILITY COMPLEX GENOTYPES ENABLES SEMI-QUANTITATIVE EVALUATION OF GERM CELL TRANSPLANTATION EFFICACY IN *JAPANESE FLOUNDER*

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Germ cell transplantation has many potential applications in fish aquaculture, particularly in the production of fish species with genetic backgrounds and phenotypes that are desirable for cultivation (Farlora et. al. 2014*). Germ cells, which are injected intraperitoneally into recipients, move spontaneously to the genital organs where they mature into spermatozoa and oocytes. To evaluate whether the germ cells have been transplanted and differentiated to functional gamates successfully, PCR can be used to detect the donor genotype that appears in the recipient germ cell. However, PCR is qualitative, and has relatively low sensitivity due to the amplification bias that prefers amplifying a major genotype and suppressing a minor one. Furthermore, finding a nucleotide polymorphism that is suitable for PCR analysis and capable of distinguishing between donors and recipients can be difficult. We performed germ cell transplantation in *Japanese flounder* and assayed both donor and progeny genotypes for polymorphisms by next generation sequencing (NGS) and conventional PCR. We selected the major histocompatibility complex (MHC) alpha/beta genes to distinguish between donor and recipient genotypes as they are highly polymorphic. We obtained a million MHC sequences and could easily distinguish between donor and recipient genotypes. NGS is well suited to detecting small genomic differences, such as single-nucleotide polymorphisms, and to quantitatively estimate the proportion of successfully transplanted genotypes. Conversely, even though PCR is technically more straightforward, the sensitivity of PCR is lower and it is less quantitative. Here we provide several examples of how NGS is capable of detecting transplantation at the 1% level and show how digital MHC genotyping by NGS is effective for assaying germ cell transplantation. We also report a protocol for such experiments and provide typical results.

*Farlora et al., Intraperitoneal germ cell transplantation in the Nile Tilapia *Oreochromis niloticus*, Mar Biotechnol (2014) 16:309-320

Keywords: germ-cell transplantation, next generation sequencing, Major histocompatibility complex, polymorphism

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THE MYSTIFYING DIVERSITY OF IMMUNOGLOBULIN M HEAVY CHAIN GENES AND ITS REPERTOIRES IN BIGHEAD CATFISH (*CLARIAS MACROCEPHALUS*)

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Abstract

The bighead catfish (*Clarias macrocephalus*) is the most important species used for producing the hybrid catfish (*C. macrocephalus* x *C. gariepinus*), one of the commercially inland fish in Southeast Asia. However, the bighead catfish is generally found to be more susceptible to infectious diseases than other fish species. To understand the key hallmarks of the catfish adaptive immune system, full-length cDNAs encoding the IgM heavy chain genes and their gene counterparts of the bighead catfish were intensively cloned and characterized. Interestingly, the results revealed that the bighead catfish possesses 4 different subtypes of membrane bound IgM molecules (mIgMA-mIgMD), which contain VDJ-Cμ1-Cμ2-Cμ3-TM1-TM2 similar to other teleost mIgMs and a single secrete form (sIgMA) with a normal VDJ-Cμ1-Cμ2-Cμ3-Cμ4 pattern. Moreover, three novel patterns including VDJ-Cμ1-Cμ2-Cμ3-Cμ4-TM1-TM2 similar to those tetrapod mIgMs, and VDJ-Cμ1-Cδ2-Cδ3-Cδ4-Cδ5-Cμ3-TM1-TM2 and VDJ-Cμ1-Cμ2-Cμ3-CμX-Cμ4-TM1-TM2 (never identified in any vertebrates) have been firstly discovered in the bighead catfish. Additionally, structural analysis of 150 cDNAs encoding variable domains of the IgM heavy chain revealed that at least 10 V_H families, 15 D_H segments and 12 J_H families were utilized using several mechanisms to generate the repertoire of antigen-binding domains. Variation analysis of the variable domains using Wu-Kabat and Shannon entropy analyses indicated that the amino acid sequences of the framework regions (FRs) were less variable than those of the complementarity determining regions (CDRs), among which the most variable was CDR3. Diversity analyses of high variabilities on variable domain of IgM obviously indicate several mechanisms used to diversify the molecules to specifically bind to various antigens. The obtained data from the present study are important key information to our understanding of the evolution and adaptive immune system of the bighead catfish.

Keywords: Bighead catfish, Immunoglobulin M, heavy chain genes, diversity

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THE OSSIFICATION MODE AND GENETIC REGULATION OF INTERMUSCULAR BONES IN TELEOST

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Abstract

Intermuscular bone (IB), which occurs only in the myosepta of lower teleosts, is attracting more attention because they are difficult to remove and make the fish unpleasant to eat. Most of the freshwater aquaculture fishes around the world, especially Cyprinidae species, possess a certain amount of IBs. The production of fish species, which have IBs, accounts more than 50% of whole world aquaculture production. Therefore, the breeding of Cyprinidae species without IBs will contribute a lot to the Chinese as well as world aquaculture industry.

By gaining a better understanding of the ossification process and genetic regulation of IB development, the following studies was performed. (1) Investigation of ossification patterns of IBs in different fish species, including *Megalobrama amblycephala*, *Misgurnus anguillicaudatus*, *Monopterus albus* and *Danio rerio*, indicated that the ossification of IBs might be related to fish swimming mode, and their morphological polymorphism maybe correlated to fish swimming mode and body type. (2) The integrated analysis of histological (Alcian blue and alizarin red S staining)-transcriptomic-proteomic demonstrated that IBs were undergoing intramembranous ossification without a cartilaginous phase. (3) Using comparative transcriptomics, small RNA omics, proteomics and genomics, a batch of genes including BMP family genes, SCX, MKX, tnmd, xirp2a, runx2a/b, osterix, entpd5, and so on, were screened out and their regulation roles during the developmental process of IBs were investigated. The phenotypes of strains from several genes knock-out through CRISPR/Cas9 technology were investigated. (4) The genetic parameters of the number of IBs in blunt snout bream were evaluated using full-sib families, which indicated the heritability of number of IBs is moderate. The BLUP values were estimated and the good families with less IBs number were selected.

Keywords: teleost; intermuscular bones; ossification pattern; gene regulation; breeding

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CORTISOL ACTING THROUGH THE GLUCOCORTICOID RECEPTOR IS NOT RESPONSIBLE FOR EXERCISE-ENHANCED GROWTH BUT DOES AFFECT THE WHITE SKELETAL MUSCLE TRANSCRIPTOME

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Abstract

Forced sustained swimming exercise at optimal speed enhances growth in many fish species, particularly through hypertrophy of the white skeletal muscle. The exact mechanism of this effect has not been resolved yet. To explore the mechanism, we first subjected wild-type zebrafish to an exercise protocol validated for swimming-enhanced growth (Fig. 1), and showed that exercised zebrafish, which indeed showed enhanced growth, had higher cortisol levels than the non-exercised controls. A central role was therefore hypothesized for the steroid hormone cortisol acting through the Glucocorticoid receptor (Gr). Second, we subjected wild-type zebrafish and zebrafish with a mutant Gr to exercise at optimal, suboptimal and super-optimal speeds and compared them with non-exercised controls (Fig. 1). Exercised zebrafish showed growth enhancement at all speeds, with highest growth at optimal speeds. In the mutant fish, exercise resulted in growth enhancement similar to wild type zebrafish, indicating that cortisol cannot be considered as a main determinant of exercise-enhanced growth. Finally, the transcriptome of white skeletal muscle tissue was analysed by RNA sequencing. The results of this analysis showed that in the muscle tissue of mutant fish a lower number of genes is differentially regulated by exercise than in wild type fish (494 versus 762). A cluster of 110 genes was regulated in both wild type and mutant fish. In this cluster, genes involved in transcriptional activity and protein ubiquitination were overrepresented. Since growth was enhanced similarly in both wild type fish and mutants, these processes may play an important role in exercise-enhanced growth.

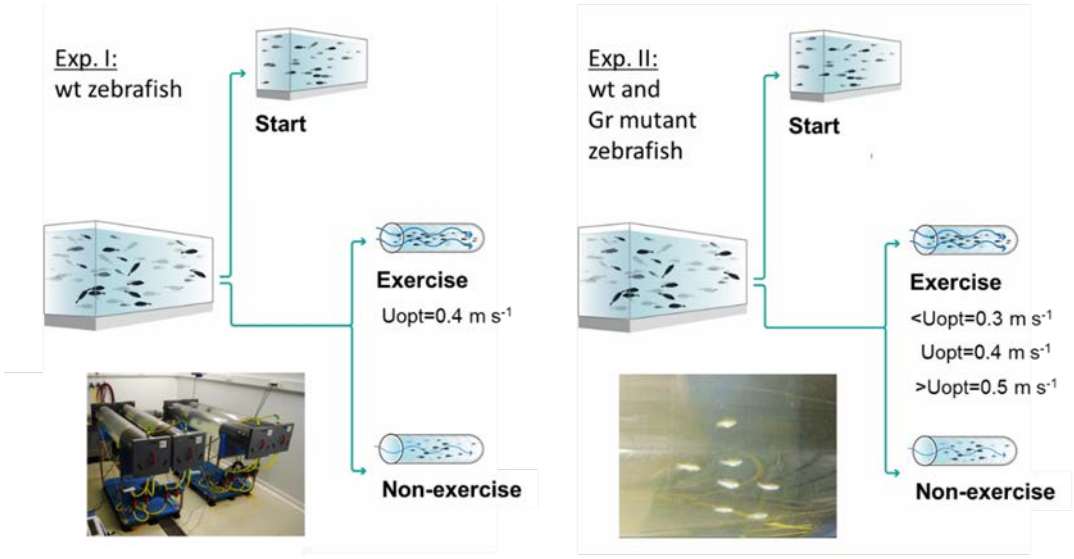

Figure 1: Experiment I was executed with wild type (wt) zebrafish with a control group sampled at the start of the experiment, a non-exercised group and an exercised group which was subjected to forced sustained swimming at the optimal swimming speed (Uopt) of 0.4 m s^{-1} for a period of 4 weeks in a swim tunnel. Experiment II had a similar set-up but was executed with wild type zebrafish and Gr mutants. Now there were three exercise groups swimming at the suboptimal (<Uopt), optimal and superoptimal (>Uopt) swimming speeds of 0.3, 0.4 and 0.5 m s^{-1} respectively.

Keywords: Swimming physiology; hypothalamic-pituitary-interrrenal (HPI) axis; cortisol; glucocorticoid receptor; RNAseq

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GENE EDITING OF CANDIDATE GENE FOR PARASITE DISEASE RESISTANCE IN YELLOWTAIL *SERIOLA QUINQUERADIATA* AND ARTIFICIAL INFECTION TEST OF *BENEDENIA SERIOLAE*.

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The gene locus conferring resistance to *Benedenia* disease in yellowtail was identified by QTL analysis, and correlation analysis using wild fish with candidate genes in the locus region strongly implicated the C-type lectin gene in resistance to *Benedenia*（Nakamoto et al., 2017）. Here, we used genetic editing techniques to create individuals with the suppression of gene function and promoted the development of technology for evaluating gene function using the *Benedenia* infection test. To produce genetically modified yellowtail fish, a method for the microinjection of an artificial nuclease RNA into a fertilized egg was established and its efficiency was improved. We also developed a hatching management method for a small number of eggs obtained from microinjected eggs. In this study, we report on the results of genetic editing of the F_0 population that will become the founders for fish for a function inhibition test of the *Benedenia* resistance candidate gene, as well as the results of the artificial *Benedenia* infection test conducted using the test fish. The efficiency of creating F_0 individuals with editing of the *Benedenia* resistance candidate gene was 68.3% at 6 weeks of age after fertilization. Gene editing was confirmed in each tissue (fins, liver, gonads, blood). A parasitic test of *Benedenia* was conducted to investigate the difference in the number of parasites. The results revealed no significant difference in the infection of *Benedenia* among the gene editing F_0 group, the nonediting group, and the control group. It was thus shown that partial reduction in the function of candidate genes may not affect parasitism of *Benedenia*.

This work was supported by the Technologies for Creating Next-generation Agriculture, Forestry, and Fisheries (funding agency: Bio-oriented Technology Research Advancement Institution, NARO) for yellowtail short-term breeding program.

Keywords: Yellowtail; Gene editing; Seriola quinqueradiata; Benedenia seriolae

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UNDERSTANDING THE MACRO- AND MICRO-EVOLUTION OF BIVALVES: INSIGHTS FROM TWO SCALLOP GENOMES

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Abstract

Bivalve molluscs, which first appeared in the early-Cambrian over 500 million years ago, represent an ancient and evolutionarily successful lineage of bilaterians that has survived several mass extinction events. Despite their great evolutionary and biological significance, our sampling of their genomes remains very limited. Recently, our group has finished the whole-genome sequencing of two scallop species, *Patinopecten yessoensis* (Nat. Ecol. Evol. 2017) and *Chlamys farreri* (Nat. Commun. 2017). Analysis of the *P. yessoensis* genome and extensive transcriptomes reveals outstanding preservation of ancestral bilaterian linkage groups, an intact Hox gene cluster under new expression control and diverse phototransduction cascades with a potentially ancient *Pax2/5/8*-dependent pathway for noncephalic eye formation, providing insights into the evolution of genome organization and developmental control during the emergence of bilaterians. Our multi-omic analyses of *C. farreri* revealed novel genomic features and molecular changes that may underlie aspects of the scallop's adaptation to semi-sessile and filter-feeding life including the well-developed adductor muscle, sophisticated photoreception system, rapid byssal production, and remarkable resistance to potent neurotoxins, suggesting that expansion and mutation of those genes may have profound effects on scallop's phenotype and adaptation. The two scallop genomes provide valuable resources and expand our understanding of the macro- and micro-evolution of bivalves.

Keywords: bivalve molluscs, genome, evolution, molecular adaptation

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APPLICATION OF GENOME EDITING IN AQUACULTURE: IDENTIFYING AND EDITING THE GENE DETERMINING GOLDEN COLOUR IN TILAPIA

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Abstract

The recently developed genome editing technology clustered regularly interspaced short palindromic repeats associated with Cas9 (CRISPR/Cas9) has enabled us to perform efficient and precise targeted genome editing in different species. However, its application in aquaculture is still in its infancy. In this presentation, I will briefly review genome editing technologies and show an example in editing a gene determining the golden colour in tilapia, which was identified and cloned by positional cloning.

We found a natural recessive golden mutation in Mozambique tilapia. Using linkage mapping and positional cloning, we found that the PMEL was responsible for golden and black colors. Further detailed analysis the sequence of the gene in black and golden individuals revealed that an inserted hairpin structure in 3′ UTR of the gene is responsible for the golden color. Expression analysis in vivo and in vitro showed that the hairpin structure reduced the transcripts of the PMEL gene. Knockout of the gene with CRISPR/Cas9 in black tilapia resulted in golden coloration, and rescue of the gene in golden tilapia recovered the black color. Our finding uncovered the genetic mechanism of golden color in teleost fish for the first time, and indicates that the hairpin structure in 3′ UTR involves in the transcriptional regulation in eukaryotes.

Key words: Aquaculture, genetic improvement, cloning, genome editing

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DE NOVO ASSEMBLY, CHARACTERIZATION, FUNCTIONAL ANNOTATION AND EXPRESSION PATTERNS OF THE BLACK TIGER SHRIMP (*PENAEUS MONODON*) TRANSCRIPTOME

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Abstract

The black tiger shrimp (*Penaeus monodon*) remains the second most widely cultured shrimp species globally; however, issues with disease and domestication have seen production levels stagnate over the past two decades. To help identify innovative solutions needed to resolve bottlenecks hampering the culture of this species it is important to generate genetic and genomic resources. One such use of genomic resources is that of genomics-assisted breeding, which can benefit from a well annotated genome; however, the successful annotation of a draft genome requires corresponding transcriptomic data, especially at the isoform-level. In a first step, we have produced the most complete publicly available *P. monodon* transcriptome database to date based on Illumina short-read data of nine adult tissues and eight early life-history stages (BUSCO - Complete: 98.2% [Duplicated: 51.3%], Fragmented: 0.8%, Missing: 1.0%). The short-read based assembly resulted in 236,388 contigs, which were then further segregated into 99,203 adult tissue specific and 58,678 early life-history stage specific clusters. While annotation rates were low (approximately 30%), as is typical for non-model organisms, annotated transcript clusters were successfully mapped to several hundred functional KEGG pathways. Transcripts were then clustered into groups within tissues and early life-history stages, providing initial evidence for their roles in specific tissue functions, or developmental transitions. In a second step, the tissue and early life-stages were sequenced with PacBio long-read technology in order to generate improved isoform-level information. Together, it is expected that this transcriptome information will provide an essential resource to investigate the molecular basis of commercially relevant-significant traits in *P. monodon* and other shrimp species.

Keywords: long-read sequencing, short-read sequencing, differential gene expression, splice-variants

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OPTIMIZATION OF TRIPLOIDY INDUCTION IN BENNI (*MESOPOTAMICHTHYS SHARPEYI*), USING HEAT AND COLD SHOCKS.

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Abstract

Chromosome manipulation produce triploidy, which is expected to improve fish yield in aquaculture. A study was done to identification of optimum temperature for triploidy induction in *Mesopothamichthys sharpeyi* by altering the duration and timing of application of heat and cold shocks. Heat shocks (34, 36 and 38 °C) and cold shocks (2 & 4 °C) were used for 3 and 5 minutes on recently fertilized eggs in two intervals (2 & 5 minutes after fertilization) which formed a total of 24 treatments in three replicates in addition to the control group, which developed without any shocks. Eggs were incubated under hatchery conditions at 23°C. The highest triploid yield was obtained in 34°C, 2 & 5 minutes after fertilization which lasted for 5 minutes. In triploid individuals, the indices of the nucleus increased significantly, confirming that the use of the RBC method could be used as a diagnostic method for the polyploidy state. The results of this study showed that induction of triploidy by thermal shocks especially heat, can lead to the production of triploid individuals in Benni. Regarding this result, in order to investigate the effect of triploidy on growth status and its comparison with diploid individuals it is recommended to continue the culture process until the maturity stage for future studies.

Keywords: Barbus, Benni, Heat shocks, polyploidy

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ANDROGENETIC DEVELOPMENT IN FISH - RADIATION-INDUCED ALTERATIONS IN THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) EGGS

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Abstract

Ionizing radiation (IR) is applied to inactivate nuclear genome in the rainbow trout eggs during androgenesis. However, it has been considered that doses of IR used to damage maternal chromosomes may also affect maternal RNA deposited in the cytoplasm during oogenesis and lead to the post ovulatory oocyte aging. To verify this assumption, quality and quantity of RNA extracted from the irradiated rainbow trout eggs were studied using standard molecular techniques and RNA-Seq approach. Moreover, distribution of lipid droplets in the irradiated and non-irradiated rainbow trout eggs and morphology of larvae that hatched from androgenetic and control groups were compared. Eggs were irradiated 350 Gy of X-rays, inseminated and exposed for the high hydrostatic pressure (HHP) shock to develop as androgenetic doubled haploids (DHs). RNAs from irradiated and non-irradiated eggs were extracted and their integrity evaluated and compared. A total of 800 ng of purified RNA was used as input for TruSeq RNA Sample Prep v2 kit (Illumina). Validated and normalized libraries were sequenced using TruSeq SBSv3 Sequencing kit (Illumina). No significant differences in RNA quantity were observed between control and irradiated eggs. Nevertheless, minor transcriptome alterations in the irradiated and non-irradiated eggs were shown. Irradiation of rainbow trout eggs with 350Gy altered transcripts of 754 genes analyzed on a pointwise level ($p<0.05$). On a genome-wide level, statistically significant ($p<0.01$) differences between irradiated and non-irradiated rainbow trout eggs concerned only few transcripts. In irradiated eggs, transcript of *nmrk2* (nicotinamide riboside kinase 2) gene showed reduced expression level while transcripts of genes known as "immediate early genes"; *ier2* (immediate early response 2) and *egr1* (early growth response 1) were found to be upregulated. Most of the non-irradiated eggs exhibited rather equal distribution of the oil droplets whereas eggs studied after irradiation had coalesced lipid droplets, a pattern found in the eggs with reduced quality. Incidences of abnormally developed larvae were more frequently observed among fish that hatched from the irradiated eggs. Provided results exhibited irradiation of rainbow trout eggs in order to inactivate maternal chromosomes may also affect maternal RNA and lead to rapid post ovulatory oocyte aging.

Key words: androgenesis, lipid droplets, maternal RNA, larvae, transcriptome

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JAPANESE FLOUNDER: GENOME TO GENOMIC SELECTION BREEDING

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Abstract

Japanese flounder (*Paralichthys olivaceus*) is an economically important cultured marine flatfish, which has the most extreme asymmetric body morphology of vertebrates. To study the role of genomic architecture in asymmetry development during flatfish metamorphosis, we produced a high-quality reference genome (546 Mb) of the Japanese flounder, based on 52.6 Gb of high-quality Illumina sequencing data. The contig and scaffold N50 sizes was 30.5 kb and 3.9 Mb, respectively. Comparative genomics between flounder and Chinese tongue sole (*Cynoglossus semilaevis*), and transcriptomic analyses during metamorphosis revealed that thyroid hormone and retinoic acid signaling, as well as phototransduction pathways may play a role in metamorphosis. We demonstrated that retinoic acid is critical in establishing asymmetric pigmentation and, via cross-talk with thyroid hormones, in modulating eye migration. Moreover, the unexpected expression of the visual opsins from the phototransduction pathway in the skin translates illumination differences and generates retinoic acid gradients that underlie the generation of asymmetry. In aquaculture of Japanese flounder, diseases caused by bacteria and virus occur frequently and resulted in huge economic loss. Genomic selection is a promising technique for selective breeding with superior strains. To perform the genomic selection in the Japanese flounder, we have constructed approximately 300 families. A total of 931 individuals from 90 families were selected and challenged with *Edwardsiella tarda*, a major pathogenic bacteria, in 2013-2015 and their survival rates were recorded as the phenotypic traits. We performed genome resequencing for these individuals (931) and their parents (71), and obtained 1,934,475 SNP markers. Two different algorithms, BayesCπ and GBLUP, were used for calculation of GEBV (genome estimated breeding value), respectively. According to the survival rates after infection, families were divided into high and low resistant groups, respectively. GEBV of different groups had a significant difference (*p-value* < 0.05), and high resistant group had a higher mean GEBV. In the selection candidates, Pearson's correlation between TBV (true breeding value) and GEBV calculated by BayesCπ and GBLUP was 0.706 and 0.795, respectively. To summarize, we have established the genomic selection method and applied it in selection of Japanese flounder with enhanced disease resistance.

Keywords: genome sequencing, metamorphosis, genome selection, disease resistance, Paralichthys olivaceus

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