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Current status of research on aquaculture genetics and genomics-information from ISGA 2018

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ABSTRACT

The ISGA (International Symposium on Genetics in Aquaculture) is an important conference in the field of aquaculture genetics, which is held every three years. This year, the meeting was hosted by James Cook University, in Cairns. The conference sessions included biotechnology and functional genomics, sex control, genomic prediction, selective breeding and quantitative genetics, industrial use of genetics, genome technology, genetics of diseases and stress, genetics of nutrition, epigenetics, genomics and metabolomes, population genetics and ethics, food safety, as well as environmental risks. Many new and interesting unpublished data, including those for new aquaculture species, marker-assisted selection, genomic selection and genome editing using Crispr/Cas9 technologies, were reported at the conference. In this brief review, we highlight the novel and interesting researches on aquaculture genetics and genomics to give readers an overview of the current status and trends of aquaculture genetics and genomics research.

1. Introduction

The ISGA (International Symposium on Genetics in Aquaculture) is an important conference in aquaculture genetics and genomics, which is held every three years. This year, the ISGA XIII was held on 15–20 July in Cairns, Australia. Over 220 people from more than 30 countries attended the symposium. The participants presented the current advances in novel technologies, including whole genome sequencing of aquaculture species, genotyping by sequencing (GBS), Crispr/Cas9 technologies, QTL mapping, genome-wide association study (GWAS), marker-assisted selection (MAS) and genomic selection (GS), sex control, as well as issues in aquaculture, demonstrating the tremendous breadth of research activities in aquaculture genetics, genomics and breeding. This symposium provided a valuable forum to learn from researchers working on different fish species, as well as exchange information and ideas, on how to accelerate genetic improvement of aquaculture species through traditional genetics, biotechnology, MAS, GS and through the integration of these technologies. Here, we review the highlights of the conference and provide perspectives on the future of aquaculture genetics, genomics and breeding. More detailed information about the presentations, abstracts and posters can be found in the symposium website: https://www.jcu.edu.au/international-symposium-of-genetics-in-aquaculture/our-committee.

2. Established and new aquaculture species

Aquaculture production has increased rapidly during the last three decades. This is due to the increased production of established aquaculture species and a continuous introduction of new ones. At this symposium, Dr. Graham Mair, representing FAO (Food and Agriculture Organization), presented “The state of the world's aquatic genetic resources” (Mair et al., 2018). According to him, there are a total of 694 aquaculture species/species items in the world, including over 250 which have not previously been reported to FAO with their production statistics. The most species are farmed in Asia, while North America farms the fewest. The two most commonly reported species are common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*). Nine of the ten most widely cultured species, namely common carp, Nile tilapia, rainbow trout (*Oncorhynchus mykiss*), grass carp (*Ctenopharyngodon idella*), Pacific white shrimp (*Litopenaeus vannamei*), African sharptooth catfish (*Clarias gariepinus*), giant freshwater prawn (*Macrobrachium rosenbergii*), bighead carp (*Hypophthalmichthys nobilis*)

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and other tilapia species (*Oreochromis* spp), are farmed in more countries where they are non-native than in countries where they are native. In several sessions of the symposium, several new aquaculture species were reported. These species include ballan wrasse (*Labrus bergylta*), lumpfish (*Cyclopterus lumpus*), dusky kob (*Argyrosomus japonicus*), yellowtail amberjack (*Seriola lalandi*) (Martínez, 2018) and the tambaqui (*Colossoma macropomum*) (Nunes et al., 2018). Ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*) are cleaner fish, which are a popular biological control solution to manage sealice infestations in commercial Atlantic salmon (*Salmo salar*) farming (Selly et al., 2018). Dusky kob is a silvery to bronze-green coloured fish, a member of the *Sciaenidae* family, which grows up to 2 meters in length. This fish has been identified as an emerging aquaculture species in South Africa due to its high market value (Jenkins & Rhode, 2018). The southern yellowtail amberjack, also called great amberjack, is a large fish found in the Southern Ocean. This fish was being developed for aquaculture in Chile (Martínez, 2018). The tambaqui is a large species of freshwater fish in the family *Serrasalmidae*. It is native to tropical South America, but also reared for aquaculture and introduced to other countries. In this fish, six mutants, which do not have intramuscular bones, have been identified by a Brazilian group (Nunes et al., 2018). The discovery of these intramuscular boneless individuals may help develop a new variety of this species, which may increase the commercial value of this fish. Although most products of aquaculture come from a few major species (e.g. carps, tilapia, salmon, rainbow trout and catfish), it seems that aquaculture is becoming more diversified, rather than just focusing on a few major species. On the other hand, although most aquaculture scientists and FAO advocate aquaculture species diversification, further diversification is limited by culture technology, profitability, sustainability, regulations and community acceptance. To overcome these constrains on aquaculture species diversification, it is essential to invest on R &D on new aquaculture species.

3. Population genetics

Population genetics aims at gaining knowledge on allelic diversity, genetic diversity, inbreeding, as well as relationships among wild and cultured populations, for biodiversity conservation and aquaculture. At this symposium, there were 10 oral presentations on this topic. While mitochondrial DNA and microsatellites are still being used for study on population genetics, genotyping by sequencing (GBS) has become the most popular tool (Xue, Chen, Peng, Zhou, & Pu, 2018). SNP-chips have also been used to analyse the population structure of salmon (Dodds et al., 2018; Martínez et al., 2018). Although GBS offers a cost-effective approach to assaying many markers, one drawback is that alleles, rather than genotypes, are sampled. Therefore, it is unclear whether both alleles (for diploid organisms) have been sampled when all reads give the same base at a position.

Dr Dodds from New Zealand investigated methods, which calculate measures of population structure by modelling the allele sampling process in GBS, thereby allowing GBS to be effectively used for population genetics studies (Dodds et al., 2018). Most researches on population genetics focused on the comparison of genetic diversity between wild and cultured populations and made insightful suggestions for aquaculture and breeding of the species of interest. It is anticipated that population genetics will continue playing an important role in aquaculture and in gaining novel knowledge about genetic variations existing in cultured and wild populations of species of interest.

4. Quantitative genetics and selective breeding

Quantitative genetics continues playing an important role in selective breeding of aquaculture species. At this symposium, there were 12 oral presentations and 22 posters on this topic. Genetic parameters have been estimated for several important traits (e.g. growth, feed conversion) in several species, including Siberian sturgeon (*Acipenser baerii*), Pacific white shrimp, abalone species, tilapia and other species. One interesting study on the estimation of the heritability of the feed conversion rate (FCR) in tilapia was reported by a French group (Verdal, Vandeputte, Mekkawy, Chatain, & Benzie, 2018). They studied 1000 juvenile fish, reared in groups, from 40 pedigreed families of the GIFT (Genetically Improved Farm Tilapia) strain of Nile tilapia, using video assessment, to evaluate the feed intake of individual fish during 13 consecutive meals over seven days and to estimate the genetic parameters of growth traits, feed intake, FCR and residual feed intake. In particular, the heritability for feed intake and FCR in tilapia was estimated to be 0.45 ± 0.09 and 0.32 ± 0.11, respectively. Their data indicates that the heritability of FCR in tilapia is intermediate, suggesting phenotypic selection of FCR could be effective. It is obvious that conventional selective breeding with the assistance of quantitative genetics is still, and will remain as, the major approach for genetic improvement of salmon, shrimp, tilapia and other major aquaculture species. New technologies, including MAS, GS and genome editing (GE) (Bastiaansen, Megens, & Komen, 2018), are being integrated into selective breeding programs to accelerate genetic improvement and introducing of new traits.

5. Genome technologies and biotechnologies

Genome technologies and biotechnologies have been applied in studies on growth, disease and stress resistance, reducing intramuscular bones, as well as evolution of species. Genome sequencing and analysis of transcriptomes are still the major approaches for generating genomic resources for genetic studies. One of the most interesting developments is the application of genome editing in generating new traits. The plenary speaker Dr. Kleppe, from Norway, gave an overview of the status of genome editing on salmon (Kleppe et al., 2018). Researchers from Norway have established a protocol for gene knock out by Crispr/Cas9 in Atlantic salmon, and are currently exploring ways to perform gene knock in. They have been successful in using Crispr/Cas9 to knockout one single gene, dead end (dnd). They are also using Crispr to identify the causative mutation for later sexual maturation both by knockout of genes in the region where QTL controlling the age of puberty onset was identified and by homologous recombination experiments using clonal lines with either genotypes for the late and early maturation alleles. Professor Yue from Singapore presented a story on identifying and editing the gene determining golden colour in tilapia (Yue, Liu, & Sun, 2018). His lab has successfully identified the gene (*PMEL17*) determining the black and yellow colours in Mozambique tilapia through positional cloning. They confirmed that this gene is the causative gene for the black and golden colours using Crispr/Cas9 technology. An insertion in the 3′ UTR of the *PMEL17* gene reduced its expression, which inhibited the transport and accumulation of pigments in the skin. Their study also showed that Crispr/Cas9 technology could be used to generate preferred phenotypes. Dr. Okamoto from Japan presented a very interesting idea in controlling an invasive species, bluegill (*Lepomis macrochirus*) (Okamoto et al., 2018). They are working on the development of a gene suppression method that releases males with sterilization genes whose functions are deficient in female-specific reproduction-related genes (i.e. modified males will mate with other fish, and the offspring will include females which are deficient in reproductive function). To inhibit the functions of *fshr* and *foxl2*, which are expressed in the female gonads and are important for sexual differentiation and maintenance of ovarian function, they produced gene-edited F0 individuals using the Crispr/Cas system. They obtained three fish, which were knocked out for both *fshr* and *foxl2*. These F0 edited individuals are currently being developed as parent fish for the creation of F1 individuals. Professor Bao's group from the Ocean University of China introduced a novel GBS (Genotyping by sequencing) method (Zeng, Shi Wang, Dou, Liu, & Bao, 2018), called Multi-isoRAD, which is an upgraded method of 2b-RAD genotyping. Their method allows the preparation of five concatenated restriction site-associated DNA tags for

Illumina paired-end (PE) sequencing. The configuration of the five concatenated tags can be flexibly designated to meet various research purposes. This method is simple and cost-effective and opens up a way for GS in aquaculture breeding. Dr. Bastiaansen from the Netherlands presented an interesting study on genome editing (GE) to introduce new traits into aquaculture breeding populations (Bastiaansen et al., 2018). They investigated the impact of GE in bringing a new trait, a disease resistance allele, into a population, and found out that with GE success of 1%, the frequency of the desired allele reached 1%, 5% or 16%, respectively, with 6, 30 or all families edited. With 10% success, allele frequency reached 10%, 34% or 89% respectively. With moderate selection for the desired allele, fixation (> 99% frequency) was reached after 13, 10 or 8 generations if there was 1% GE success and after 9, 7 or 6 generations if there was 10% GE success. Hence, with low GE success, selection is needed to effectively introduce a new trait. Without selection for the introduced disease resistance allele, many GE procedures are needed and desired allele frequencies will remain low for many generations.

It is evident that genome technology and genome editing are being intensively used to improve economically important traits in some countries. Surely, genome editing will make a big impact on aquaculture breeding programs too. Currently, in aquaculture species, there are not many genes with known functions in determining the phenotypes. Furthermore, most economically important gene are quantitative in nature and are determined by many genes, environmental factors and their interactions. Therefore, knockout of single gene may not affect the phenotypic values significantly. In the future, more of such causative genes can be identified in aquaculture species, through methods such as QTL mapping, GWAS and positional cloning, allowing the thus-identified genes to be modified to improve the corresponding traits. Knowledge about gene functions from model organisms is also useful in obtaining genes to manipulate.

6. Marker-assisted selection (MAS) and genomic selection (GS)

After over 30 years of extensive research on developing genomic resources (e.g. DNA markers, genome and transcriptome sequencing), conducting QTL mapping, and recently GWAS due to the rapid reduction in cost of genome sequencing and genotyping, MAS and GS are being applied to breeding programs in some species. In this session, Dr Ben Hayes from Australia gave a plenary presentation on “Genomic selection and its revolutionary application to aquaculture genetic improvement” (Hayes, 2018). He showed that more than three million livestock have been genotyped with SNP arrays for the purposes of genomic selection. In dairy cattle, the technology has doubled the rate of genetic gain. He pointed out that future increases in accuracy of phenotype predictions may come from combining genome and commensal microbiome profile information. Some data of QTL mapping and GWAS are being used in MAS and GS in salmon (Dagnachew, Meuwissen, & Sonesson, 2018), rainbow trout (Liu, 2018), Japanese flounder (Chen, 2018) and other species. In rainbow trout, MAS based on a few DNA markers associated with traits of interest can reach high selection accuracy (> 0.5), suggesting that for MAS, it is not necessary to use many markers if the effect of QTL is reasonably large. Dr. Dagnachew from Norway presented an interesting story on use of DNA pooling in genomic selection for a disease resistance trait in Atlantic salmon (Dagnachew et al., 2018). The study by his team showed that DNA pooling seems to be a good strategy to reduce the cost of conducting GWAS without reducing the prediction accuracy (Dagnachew et al., 2018).

So far, most GWAS were conducted based on a training population with less than 1000 individuals, thus significantly limiting the prediction powers of the SNPs when applied to GS for the species of interest in general. Ideally, GWAS should be conducted with over 1000 individuals from many different families and populations. Cost-reduction strategies such as that proposed by Dr. Dagnachew can hopefully encourage the use of larger and more varied study populations in future GWAS.

Although there are a few examples in GS in some species, including salmon and rainbow trout, there is no detailed information about the economic benefits of GS in any aquaculture species. It seems that the cost of genotyping of a large number of SNPs and many individuals are still a major obstacle for GS in aquaculture. Further reducing the cost of whole genome genotyping to less than $10/individual, is essential to make GS, economically viable in aquaculture breeding programmes.

7. Sex control and its applications

Sex control is a hot topic in aquaculture genetics. In the symposium, there were 14 oral presentations and five posters on this topic. Professor Piferrer from Spain gave an interesting plenary talk on “Development of essential epigenetic markers: application to the prediction of gonadal sex and the identification of early signs of domestication”. He presented a conserved model of epigenetic regulation of sexual development in fish (Piferrer, 2018). The model assumes the existence of “pro-male” and “pro-female” genes and the canonical inverse relationship between gene silencing and gene expression levels, and it deals with the relationship between gene silencing states and gene expression levels during sex differentiation in gonochoristic species or sex change in hermaphroditic species. Other oral presentations in this session presented potential sex determining genes in some species. For example, in turbot, the sex determining locus was mapped on LG5 in a 531 kb region where the master gene is putatively located (Martínez et al., 2018). The potential gene is a new gene, and a noncoding RNA might play a role in the sex determination. In channel catfish, the sex determining gene was identified as BCAR1 (Bao et al., 2018). Professor Hende Li from CAFS, China, presented an interesting mechanism underlying sex reversal in the half-smooth tongue sole (*Cynoglossus semilaevis*). They found that two SNPs, Cyn_Z_6676874 and Cyn_Z_8564889 on Z chromosome, were associated with sex reversal. The two SNPs are located in the third intron of the FBXL1 gene and in the third intron of the Dmrt1 gene, respectively (Li, Cui, & Wang, 2018). The genetic females simultaneously carrying the T allele of Cyn_Z_6676874 and the A allele of Cyn_Z_8564889 change into pseudomales. The pseudomales cannot produce W sperm. Their findings are beneficial to improving the female percentage in tongue sole aquaculture. Dr. Tom (2018) from Israel introduced a method to produce monosex giant freshwater prawn (*Macrobrachium rosenbergii*). Their method included the following three steps: (1) a single injection of androgenic gland cell suspension caused fully functional sex-reversal of females into ‘Neo-males’ bearing the feminine WZ genotype; (2) crossing Neo-males with normal females (WZ), yielded progenies containing ~25% WW females as validated by sex-specific DNA markers and, finally, (3) crossing WW females with normal males (ZZ) gave rise to all-female progenies. All-female cultures showed better performance in all parameters, including higher survival rate, higher yield per hectare and uniform body size, leading to more marketable animals from a given pond. Also, the fact that no males or berried females were found in the all-female ponds reflects the reliability of this novel technology in achieving sustainable monosex female prawn aquaculture. Although the topic of sex control is very hot, and many labs are working on that, most of these thus-identified potential sex-determining genes have not been experimentally confirmed. Perhaps, in the next symposium, more novel results on these genes will be presented. From these data presented at the symposium, evidently, the mechanisms underlying sex determination in fish are much more complicated than that in mammals. It is expected that more different sex determining genes will be identified in different aquaculture species. Understanding more about the mechanisms underlying sex determination will enable effective manipulation of the sex of aquaculture species, thus improving the productivity of aquaculture.

8. Hologenomics and its application

Hologenomics is the omics study of hologenomes. A hologenome is a whole set of genomes in an organism or ecological niche, including microbes, other life forms, and viruses. Hologenomics may include research on the interaction between an animal's genome and the genomes of gut microbes or viruses linked to the animal. Although this exact concept was not mentioned at the symposium, recently started studies, on closely related issues in aquaculture species, were presented. Prof Jerry's group (Villamil, Huerlimann, Condon, Maes, & Jerry, 2018) from Australia studied the relationships between pond yield of tiger shrimp (*Penaeus monodon*) and microbiota. They found significant differences between ponds with low and high productivity, in terms of the bacterial assemblages in the water, gut and gastric mill (GM) of shrimp. Low productivity ponds exhibited decreased bacterial diversity and richness. The gut and GM in shrimp of low productivity ponds were enriched in *Vibrio* and *Fusibacter*; cyanobacteria were enriched in the water, and the *Desulfobacteraceae* family was more abundant in sediments. They believed that these shifts in bacterial composition can serve as indicators of stress and low yielding ponds during summer months in North Queensland shrimp aquaculture. Besides this presentation, there were a few presentations on metagenomics. For hologenomic study, it is essential to simultaneously consider the holobiont (i.e. host plus its microbiome) at multiple levels, including the interactions of not only the host genome but also its epigenome and transcriptome, as well as its microbial metagenome and metatranscriptome (Limborg et al., 2018). To fully understand the functional pathways, which determine the host phenotype, studies should incorporate analyses of the associated proteomes and metabolomes, and metaproteomes and metametabolomes. In short, in the future, aquaculture breeding should consider both the host genome and its harbouring microbes to achieve the maximal genetic improvement.

9. Epigenetics/epigenomics

Epigenetics has attracted considerable attention with respect to its potential value in many areas of agricultural production, particularly under conditions where the environment can be manipulated or natural variation exists. A few studies on epigenetics and epigenomics have been reported in tilapia, rainbow trout and barramundi. In tilapia, Dr. Fernandes (Fernandes, Konstantinidis, & Podgorniak, 2018) from Norway compared the muscle methylomes and hydroxymethylomes between fast- and slow-growing full-sib Nile tilapia. Association studies between 5mC/5hmC and growth performance revealed that epigenetic regulation of growth affects several genes and biological processes, sometimes in a sex-specific manner. In female Nile tilapia, hyperhydroxymethylation of one gene of the ERM family was associated with improved growth. On the other hand, methylation levels at 63 CpG positions were significantly different between small and large fish regardless of their gender. These CpGs were mostly intergenic, but some were linked to genes involved in biological processes related to histone methylation and binding. If validated, such growth epimarkers could be used in future breeding programmes integrating epigenomic selection. It seems that the aquaculture industry has recently acknowledged the potential of epigenomic programming as a means of improving important traits. However, little is known about the mechanism underlying epigenetic regulation of important traits in fish. Future research can focus on understanding how the genome, environment and gut microbiomes interact with each other to affect the gene and phenotype expressions.

10. Issues and future directions in aquaculture genetics and genomics

Even though the oral presentations and posters presented at the ISGA 2018 did not necessarily reflect the whole picture of the current status of aquaculture genetics and genomics, a lot of new progress on all fronts of aquaculture genetics and genomics could be seen. On the other hand, a few challenging issues need to be tackled to make the progress on these fronts even quicker: 1) For selective breeding, measurement of phenotypic traits (i.e. phenomics) is critically important. Although there are some efforts to develop tools to precisely measure traits, few new tools were seen at this symposium. More research on phenomics (Houle, Govindaraju, & Omholt, 2010) should be conducted. 2) Though the cost of next-generation sequencing is decreasing rapidly, using GBS for MAS and GS is still too costly for the aquaculture industry. Further reducing the cost of GBS to less than $10/sample is essential for the application of MAS and GS in aquaculture. It is also essential to use many (> 1000) individuals in the training population for GWAS to get enough prediction power for the SNPs when applied to GS. 3) And though it is known that epigenomic programming and hologenomics are useful to aquaculture, it is not very clear how to integrate these in aquaculture. More analysis methods, including statistics, should be developed to analyse the interactions of not only the host genome but also its epigenome and transcriptome, as well as the microbial metagenome and metatranscriptome. 4) Finally, while Crispr/Cas9 is a promising tool to generate traits for genetic improvement, few genes affecting phenotypes have been found in aquaculture species. Therefore, identifying of causative genes for important traits could be an important task.

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