CONFERENCE PLENARY

GENE EDITING BY CRISPR AS A RESEARCH TOOL AND POSSIBLE APPLICATIONS IN AQUACULTURE

Kleppe L.[a], Wargelius A.[a], Skaftnesmo K. O.[a], Kjærner-Semb E.[a], Ayllon F.[a], Straume A. H.[a], Güralp H.[a], Norberg B.[b], Furmanek T.[a], Dankel D.[d], Ellingsen S.[d], Rubin C. J.[e], Hansen T.[c], Fjelldal P. G.[c], Schulz R. W.[f], Taranger G. L.[a], Andersson E.[a], Edvardsen R. B.[a]

[a] Institute of Marine Research, Bergen, Norway
[b] Institute of Marine Research, Austevoll Research Station, Storebø, Norway
[c] Institute of Marine Research, Matre Aquaculture Research Station, Matredal, Norway
[d] University of Bergen, Department of Biological Sciences, Bergen, Norway
[e] Uppsala University, Department of Medical Biochemistry and Microbiology, Uppsala, Sweden
[f] Utrecht University, Faculty of Science, Department of Biology, Utrecht, The Netherlands

Salmon farming is a key industry in Norway, with a yearly production of more than 1,2 million tons. However, sustainability concerns are currently hampering further expansion of the industry. A long-term negative environmental effect is the genetic introgression from escaped farmed salmon into wild populations. Reduced fish welfare associated with precocious maturity represents another obstacle, since robust farmed fish is needed to avoid negative stress phenotypes associated with sexual maturation, such as cataract, bone and fin deformities, higher disease susceptibility, and osmoregulatory problems.

In the recent years, the field of gene editing has made substantial advances due to the introduction of CRISPR/Cas9, a highly efficient and potent methodology. CRISPR/Cas9 allows editing of specific DNA sequences in any organism, which opens for new possibilities to edit key genetic traits in aquaculture. Using CRISPR/Cas9 allows gene editing at different levels and does not necessarily involve transgenesis, which has been the major focus for the criticism towards genetically modified organisms. With CRISPR/Cas9 it is possible to perform cisgenic editing, which does not introduce any “foreign” DNA to the organism, but instead induces changes in its existing genome sequence, which is different from traditional technologies used to edit genomes.

We have established a protocol for gene knock out, and we are currently exploring ways to perform gene knock in, by CRISPR/Cas9 in Atlantic salmon. This in combination with the sequencing of the Atlantic salmon genome allows starting a new era of improved breeding in salmonid aquaculture. Respective studies should aim at elucidating how (mono- and polygenic) traits influence for example disease resistance, reproduction, or welfare, and ultimately if and how gene edited fish can be used in aquaculture to solve major environmental bottlenecks in the production of salmon (e.g. to mitigate problems with escaped fish and diseases).

A first prerequisite for the introduction of gene edited fish to sea cage farming is sterility, to avoid any chances of genetic introgression of escapees with wild salmon populations. Therefore, we have explored the possibility to produce salmon devoid of germ cells, and thus is 100% sterile. This has been successful using CRISPR/Cas9 to knock out one single gene, *dead end* (*dnd*). Furthermore, our germ cell-free salmon shows no signs of entering puberty, which is different from the sterile triploid salmon that is currently being tested for large scale production in some salmon farms. Nevertheless, since germ cell-free broodstock cannot reproduce, we do not yet have an efficient way to produce these fishes. We are currently working on ideas on how to solve this.

Another path to control the timing of reproduction and reduce genetic introgression is to use salmon predetermined to mature late. If escaping, late maturing salmon are more likely to die before reaching the spawning grounds for wild populations. We have identified a region in the salmon genome largely controlling the age at puberty onset. Current projects are using CRISPR to identify the causative mutation both by knock out of genes in the region and by homologous recombination experiments (of putatively causative nonsynonymous SNPs) using clonal lines with either genotypes for the late and early maturation alleles.

In summary, gene editing is not only an important tool for understanding salmon biology, but we also foresee that the potential improvements considering sustainability issues may result the future use of gene edited fish in aquaculture.

KEYNOTE: GENOMIC PREDICTION

GENOMIC SELECTION AND ITS REVOLUTIONARY APPLICATION TO AQUACULTURE GENETIC IMPROVEMENT

Ben Hayes
University of Queensland

Abstract

Genomic selection is a technology which uses genome wide DNA markers, and a large reference population of individuals genotypes for these markers and phenotyped for the target trait(s), to derive prediction equations which can then be used to predict genomic estimated breeding values for selection candidates. The selection candidates can be genotyped at a very early stage in life, even as embryos, enabling rapid turnover in generations. More than three million livestock have been genotyped for SNP arrays for the purposes of genomic selection, and in dairy cattle, the technology has doubled the rate of genetic gain. Genomic selection has also been widely implemented in crops. In both livestock and crops, genomic selection is being combined with advance reproductive technologies (eg IVF in cattle and speed breeding in wheat) to further accelerate gains. Future increases in accuracy of phenotype predictions (predicting an individual's future performance) rather may come from combining genome and commensal microbiome profile information, and an example is given for feed efficiency in cattle.

KEYNOTE: GENOMIC TECHNOLOGY

APPLICATION OF GENOMICS TO ENHANCE NEW ZEALAND CHINOOK SALMON BREEDING

Walker S.P.[a§], Symonds J.E.[a], Clarke S.M.[b], Dodds K.G.[b], Blanchard B.[c], Sutherland D.[c]

[a]Aquaculture Group, Cawthron Institute, Nelson 7010, New Zealand
[b]Invermay Agricultural Centre, AgResearch, Mosgiel 9053, New Zealand
[c]Mount Cook Alpine Salmon, Twizel 7944, New Zealand

Aquaculture is an important primary industry for New Zealand and Chinook salmon (*Oncorhynchus tshawytscha*) is one of the main species farmed, currently producing close to NZD $100 million p.a. in export revenue. The three largest salmon farming companies operate in-house vertically integrated breeding programmes, including two established family based programmes now in their fourth and ninth generations, and one mass selection programme. Families are evaluated on commercial farms in mixed family groups reared together since the eyed egg or first feeding stage. Family identification relies on genotyping, traditionally using microsatellite DNA (msDNA) markers for parentage assignment. More recently, high-throughput molecular genotyping methods, coupled with efficient tissue sampling through to the bioinformatic and statistical analyses, have enabled the development of genomic tools for New Zealand Chinook salmon. Genotyping by sequencing (GBS) has proven to be a reliable and cost-effective alternative to msDNA genotyping and provides more accurate estimates of relatedness, inbreeding and genetic diversity that can be applied across programmes to better manage genetic health. We have recently completed GBS on broodstock sampled from multiple breeding programmes, reliably generating in the range of 35,000 to 46,000 SNPs per individual for analysis. GBS has been implemented within a mass selection programme to better manage matings, avoid crossing relatives and maintain diversity. The aim is also to implement genomic selection across breeding programmes to deliver within family selection benefits for difficult to measure traits and enhance genetic gain.

Keywords: Genomics, genotyping-by-sequencing, genetic health, Chinook salmon

[§] Corresponding author. Tel.: +64 3 548 2319; E-mail address: seumas.walker@cawthron.org.nz

KEYNOTE: GENETICS OF DISEASE AND STRESS

IDEAS ON DEVELOPING DISEASE RESISTANT FISH STRAINS – LESSONS LEARNED FROM COMMON CARP AND KOI HERPES VIRUS DISEASE

David, L.,

Department of Animal Sciences, R.H Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel.

E-mail: lior.david@mail.huji.ac.il

SUMMARY

Outbreaks of fish diseases inflict substantial damages on farmers and the environment and at the same time hinder sustainable production and further growth of the aquaculture sector. Since prevention and treatment of diseases in aquaculture species are less efficient than in land farm animals, there is a strong drive to develop strains genetically resistant to diseases as a sustainable solution to the problem. Yet, despite considerable efforts, the progress in developing resistant strains is limited. In my talk, I will present our key findings from developing common carp strains that are resistant to Koi herpes virus disease (KHVD). Along with presenting the breeding steps, I will discuss also what we already know on the genetic basis and mechanism of this resistance. In the light of our findings, I will further reflect on more general aspects of disease resistance genetics and breeding. I will touch upon how much and what type of variation is there for resistance, where to find it and how to utilize it. Furthermore, I will refer to what does resistance mean, how do we measure it and how can we study its genetic basis. In my view, although disease resistance is important and desirable in all farmed species, compared to land farm animals, aquaculture species offer unique opportunities to succeed in both breeding for resistance and unraveling its underlying mechanisms.

KEYNOTE: GENETICS OF NUTRITION

PHENOTYPING FOR GENETIC IMPROVEMENT OF FEED EFFICIENCY IN FISH: LESSONS FROM PIG BREEDING

Knap P.W.[a], Kause A.[b]

[a] *Genus-PIC, Schleswig, D-24837, Germany*
[b] *Animal Genetics, Natural Resources Institute Finland (Luke), Jokioinen, FI-31600, Finland*

Abstract

Feed incurs most of the cost of aquaculture production, so feed efficiency improvement is of great importance. We formulate a logical framework for assessing the most useful component traits influencing feed intake and efficiency in farmed fish – either to identify traits that can together be used for genetic improvement of feed efficiency, or as substitute traits for feed intake recording. Improvement of gross feed efficiency in growing fish can be accomplished by selection for increased growth rate. However, the correlation of growth with feed efficiency is typically only modest, and hence there is room for further improvement of feed efficiency through methods other than growth selection. Based on the meta-analysis of >40 papers, we propose that the most effective additional methods are selection for reduced body lipid content and for reduced residual feed intake (RFI). Both methods require more or less sophisticated recording equipment; in particular, the estimation of RFI requires recording of feed intake (DFI) which is a challenge. In mammals and birds, both these approaches have been effective, and despite the high costs of feed intake (FI) recording, the RFI approach has been cost-efficient because maintenance requirements are high in birds and mammals and therefore RFI variation covers a large part of FI variance. The analysis shows that maintenance requirements of fish are lower than in birds and mammals and therefore RFI variation covers a smaller part of FI variance. Moreover, accurate high-volume routine individual FI recording is much more challenging in fish than in mammals or birds. It follows that selection for reduced body lipid content is likely a more effective (and certainly more cost-efficient) way to improve FCR in fish than selection for reduced RFI. Solid evidence for these propositions is still scarce, and their generality still needs to be confirmed.

Keywords: Genetic improvement; Feed intake; Body composition; Maintenance costs; Residual feed intake

Corresponding author: Tel: +358 29 532 6222; E-mail address: antti.kause@luke.fi

KEYNOTE: EPIGENETICS

DEVELOPMENT OF ESSENTIAL EPIGENETIC MARKERS: APPLICATION TO THE PREDCITION OF GONADAL SEX AND THE IDENTIFICATION OF EARLY SIGNS OF DOMESTICATION

Piferrer F.

Institut de Ciències del Mar, Spanish National Research Council (CSIC), Barcelona, 08003, Spain

Abstract

Essential epigenetic marks (EEMs), a new concept, can be defined as the number and identity of informative epigenetic marks that are strictly necessary, albeit perhaps not sufficient, to bring about a specific, measurable, phenotype of interest. These EEMs are circumscribed to a specific temporal and spatial context, e.g., epigenetic changes that occur during sensitive periods of early development that are involved in the acquisition and maintenance of cell identity in a given tissue. Based on the identification and use of EEMs, I present the Conserved Model of Epigenetic Regulation of Sex. It is called conserved because the underlying mechanisms are independent of species and reproductive types. The model is based on the assumption that there are “pro-male” and “pro-female” genes and on the canonical inverse relationship between gene silencing and gene expression levels. This model for the epigenetic regulation of sexual development in fish, deals with the relationship between gene silencing states and gene expression levels during sex differentiation in gonochoristic species or sex change in hermaphroditic species. Importantly, it also postulates that, in terms of the epigenetic and gene expression patterns involved, the final gonadal phenotype is more relevant than the means to achieve it, be either the result of a natural process (e.g., male sex differentiation) or of human intervention (e.g., androgen-induced masculinization). Data is also presented on the use of EEMs to define the early stages of domestication. This was achieved in the European sea bass by comparing the methylome and transcriptome of somatic and gonadal tissues between wild fish and genetically similar fish reared in a farming environment. We found genome-wide differences in DNA methylation associated with the farming environment in functional gene elements. These findings show that the first steps to domestication in the absence of yet genetic changes include dynamic alterations in DNA methylation. Some of these changes are similar to genes found to be under positive selection in domesticated mammals. Our data, therefore, suggests an important role for epigenetics during the initial stage of domestication.

Keywords: Essential epigenetic marks, DNA methylation, sex differentiation, domestication.

Corresponding author. Tel. +34-932309567, Fax: +34-932309555
Email address: piferrer@icm.csic.es