FELLOWSHIP SUMMARY REPORTS

FINAL REPORT: Epigenetic markers of fish domestication for selective breeding

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Home institution: Swansea University

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Dates Fellowship: January 2022-November 2022 (postponed from 2020 due to COVID)

Consent to post the report: Yes

1. What were the objectives of the research project? Why is the research project important?

Reducing ecological footprint and disease risk are two of the main challenges faced by intensive aquaculture, which has grown exponentially over the last decades[1, 2]. Yet, in contrast to birds and mammals, relatively little has been advanced on fish domestication, which has focused almost exclusively on genetic improvements for commercial traits. However, this approach often results in inbreeding, genetic drift and loss of local adaptations. Epigenetic modifications represent a plastic response to the environment that can prepare the offspring for the conditions experienced by their parents. Epigenetic mechanisms of inheritance occur in all taxonomic groups for a variety of phenotypic traits[3, 4]. These mediate changes in gene expression and function that do not involve changes in DNA sequence [5, 6] and alter the patterns of offspring development, creating phenotypic variation that can be heritable across generations [7, 8, 9, 10]. DNA methylation is probably the best known of the epigenetic mechanisms and results in epiallelic variation which should not be subject to the limitations typically associated with genetic inheritance. For example, epimutations induced by the environment can simultaneously arise in different individuals of the population[11]. Identifying environmentally-related heritable epigenetics marks related to fitness would be a big step towards improving the adaptation of fish to farm conditions and optimising the domestication process, without strong reductions of genetic diversity. Thus, although phenotypic and genetic effects of fish domestication are apparent after just one generation[12, 13, 14, 15], the identification of specific genes associated with this process has proved elusive and genome-wide analyses have failed in identifying strong signals of selection or major genes associated with fish domestication[16, 17], and this Fellowship aimed to work on the identification of the potential epigenetic basis for these changes, an area already exploited in plant agriculture but only incipient in aquaculture[18].

Aims

The main aim of the project was to use a comparative approach to identify epigenomic signatures of domestication related to rearing density and disease resistance common to two commercial species, Atlantic salmon (*Salmo salar*) and Nile tilapia (*Oreochromis niloticus*), and to investigate their dependence on genetic diversity, with the objective of to selecting epigenetic markers that would enhance traditional selective breeding programmes.

2. Were the objectives of the fellowship achieved?

Objective 1. To identify common epigenetic markers related to rearing density and infection in Atlantic salmon and Nile tilapia (***in progress***).

Finding common epigenetic markers of disease and stress resistance between species based on our published (salmon) and unpublished (tilapia) data proved particularly challenging, with no clear differentially methylated genes

found in common between salmon and tilapia in response to stress or in response to the particular infections studied (Saprolegnia and LPS- part of the outer membrane components of gram-negative bacteria used to induce an immune response without a real infection). The results can be summarised as follows:

(a) Similar challenges between species, i.e., suboptimal density resulting in stress and infection with the same pathogen, in this case *Saprolegnia parasitica* (a fungal infection common in the farming industry for both salmon and tilapia), resulted in some similarities in gene expression changes in gills between species. In particular, genes related to innate immune response were downregulated when both species were reared under stressful conditions (high density for salon and low for tilapia), indicating a general suppression of the immune system in response to infection under particular sub-optimal density conditions.

(b) The relationship between differential DNA methylation and differential expression was not always direct (i.e., same gene affected by both). We found a large proportion of the methylation (>60%) occurring in gene bodies rather than promoters. While methylation on promoters is mainly associated with downregulation of gene expression, the result of differential DNA methylation on gene bodies is more variable. Probably for this reason, only a small number of genes (between 2-6 depending on the species and type of stressor) could be identified as both differentially methylated and expressed, none of which overlapped between species. In general, this fits well with other studies that report weak correlation between differential methylation and gene expression[19].

(c) A search of the literature was carried out to identify additional datasets of differentially methylated genes that could expand the comparisons of the results from the tilapia/salmon density/infection experiments. For this, the PRISMA protocol (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) protocol for a systematic literature review was applied[20], to identify papers with relevant epigenetics databases. A search in Google Scholar was conducted with the keywords “DNA methylation” AND “RRBS” AND “aquaculture” AND “disease” AND “Atlantic salmon”. The search was then repeated substituting “Atlantic salmon” for “Nile tilapia” and then including “stress” instead of “disease”. RRBS (Reduced representation bisulfate sequencing) was added as a search term to refine the search, as this is the most common protocol currently used to assess DNA methylation differences. The results included 74 and 43 papers for disease and salmon and tilapia respectively and 76 and 45 for Nile tilapia. Most of the papers overlapped among disease and stress (68 for salmon and 42 for tilapia), several of them had to be excluded as they were not directly working with the target species (just mentioning it in the text). While at the beginning of the study a limited number of papers (10 salmon and 3 for tilapia) contained potential databases for comparison purposes, the number has considerably increased since 21, with 24 of the 68 papers for A. salmon identified published between 2021-2023, therefore, based on the data collected here, further efforts will be dedicated to identify common markers under a new, longer project recently awarded (see below). The main obstacles in identifying common biomarkers from these datasets are (1) different tissues and life cycle stages (which naturally have different DNA methylation signatures not necessarily related to stress or disease), (b) different presentation of the raw data (in many cases it is very difficult to assign sequences to individuals and/or treatments and (c) noise derived from the particular environmental conditions of each study. Yet,

some common patterns have been recently found between key tissues like sperm[19], indicating that it is likely that we find a similar pattern in experiments carried out in similar tissue and age. The analyses of these data sets is in progress (see below).

Objective 2. To assess the degree of autonomy of epigenetic and genetic variation (***in progress***).

This objective was based on our previous resulte using a self-fertilising, highly inbred fish species (*Kryptolebias marmoratus*), indicating that, at least in part, DNA methylation patterns may be the result of a dynamic interaction between genotypes and the environment. The objective here was to identify the potential genetic basis of common markers of stress and disease but, given the results from Objective 1, we could not proceed with the analysis yet. Instead, we focused on alternative ways to identify common biomarkers:

- The results of this preliminary work indicated that the identification of epigenetic markers common to several species required more time and probably direct contact with the authors of the databases to be able to make sense of the information available. This work was included as a part of a work package of identification of epigenetic biomarkers in a Horizon Europe grant proposal which has recently being awarded (November 2022; Cure4Aqua: https://cure4aqua-project.eu). Work on this will start in October 2023.
- Sperm appeared as the most promising tissue to identify markers for several reasons, (a) it is composed of more uniform cell types than other tissues and (b) has the potential to transmit environmentally-related epigenetic modifications to the next generation. Work has been planned in collaboration with Benchmark Genetics to assess these transgenerational patterns in sperm that should start in September 2023 (Royal Society grant awarded April 2023).

3. What were the major achievements of the fellowship? (up to three)

a. Identification of common genes differentially expressed by two commercial fish species under stress conditions typical in aquaculture.

b. Identification of target epigenetic markers of domestication in fish gametes (sperm) to be explored in further research.

c. Establishment of new collaborations with the CSIC, with two grant proposals submitted awarding resolution.

4. Will there be any follow-up work?

- Is a publication envisaged? Will this be in a journal or a publication? When will it appear?

A publication is currently in progress as well as an invited talk to the workshop on Genomics and Aquaculture at the International 39th International Society for Animal Genetics Conference to be held in Cape Town, South Africa in

July 2-7, 2023 organised by Dr Maria Saura (INIA-CSIC). Submission of the manuscript expected by the end of 2024.

- Is your fellowship likely to be the start of collaboration between your home institution and your host?

It has already been, some examples of the new collaboration include:

- Collaboration in Research proposal on Disentangling the genetic basis of disease resistance in livestock and aquaculture species of economic interest using -omic tools, led by Maria Saura (Submitted).
- Proposal for Natural Resources Wales on estimations of Ne collaboration of genomic estimates of effective population size, led by S. Consuegra in collaboration with B. Villanueva and M. Saura.
- Research paper on the use of epigenetic markers for selective breeding (Consuegra, Villanueva, Saura; *in progress*).

- Is your research likely to result in protected intellectual property, novel products or processes?

No

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

Please express this in terms of environmental/food security/food safety/economic/health (human and livestock and plant) benefits, etc.

Our project has contributed to progress in understanding the mechanisms underlying trans-generational responses to food animals' rearing conditions, and the results can also be relevant to researchers interested in predicting populations' responses to natural and human-driven range shifts (e.g., those caused by climate change). Our results can help to refine current aquaculture practices in order to reduce transgenerational effects of stress, thereby helping to reduce associated productivity losses and improve welfare of the fish farming industry.

6. How was this research relevant to:

- The objectives of the CRP?

The project fitted the objective of the programme by addressing food security and sustainability. We combined two critical aspects related to sustainable aquaculture, namely domestication and disease resistance.

- The CRP research theme?

The project sits within Theme III, Transformational technologies and innovation. In particular, we used advanced tools (epigenetics/omics) to improve fish domestication, by identifying target genes affected by epigenetic modifications under farming conditions susceptible to be manipulated to increase fish resistance to stress and disease. The results of our project should contribute to sustainability, by providing new insights into the process of fish domestication and new tools to improve productivity and reduce losses, by enhancing the adaptation of fish to the farm environment.

7. Satisfaction

- Did your fellowship conform to your expectations?

Yes, it was particularly useful to start new collaborations.

- Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities? Please specify.

Yes, it has provided new opportunities to write collaborative research grants.

- Did you encounter any practical problems?

Yes, but related to COVID19, not to the Fellowship programme. The original dates for the Fellowship were between June 2020 and January 2021 and for that I had been awarded a 6 month sabbatical leave that would release me from lecture and administration duties during the time I would spend at the INIA in Madrid. However, due to COVID19-related travel restrictions, the Fellowship could not start at the planned time and the sabbatical expired before the start of the project. As a consequence, the start date had to be postponed to 2022, and the planned work had to be carried out during intermittent weeks instead of the whole 4 months initially planned as I had to carry out the Fellowship at the same time as normal lectures and University-related administrative work. At the end, I had to shorten the period of the Fellowship as it was very difficult to combine with the rest of my duties.

- Please suggest any improvements in the Fellowship Programme.

None that I can think of, I found it very useful and flexible.

8. Advertising the Co-operative Research Programme

- How did you learn about the Co-operative Research Programme?

Website.

- What would you suggest to make it more “visible”?

Perhaps social media?

- Are there any issues you would like to record?

None