Home Office

NON-TECHNICAL SUMMARY

Genetic and developmental basis of morphological variation in cichlid fish

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Evolution, Pigmentation, Cichlid fish, Genetics, Development

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of the proposed project is to identify genes responsible for differences in skin colour between species of cichlid fish. Genes are portions of the genome, which is a particle that contains all the information needed to build and maintain an organism.

Cichlids are a very diverse group of 2100 fish species, with very distinct colour patterns. In this project we will identify which genes underlie the colour diversity observed in this group of fish. More specifically, we will study body pigmentation and anal fin markings that are present only in cichlid fish males. More colour and a higher amount of anal fin markings makes males more attractive to females and increases their chances of reproduction.

To identify genes responsible for coloration differences we will test if it is possible to interbreed different species with different colour patterns using *in vitro* fertilisation methods. The genomes of the progeny with mixed colours will be sequenced to identify the genes causing colour differences. Once candidate genes are identified we will use techniques that delete and/or modify genes to study how their function affects pigmentation.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

This project will uncover genes underlying differences in colouration. This will contribute to knowledge of nature and evolution. Using techniques that delete genes, we will describe how the deletions affect skin colouration. These genes define how colours are produced both in fish and other vertebrates (e.g. birds, reptiles, mammals and humans). Taken together, we will describe the function of genes that are important to cichlid fish colouration and conclusions arising from these findings can be extrapolated to other vertebrates. Our work will be disseminated through publications in scientific journals that will be available to other researchers and the general public.

We are among the first research groups to use these gene deletion techniques in cichlid fishes, therefore the success of our project will benefit the whole cichlid research community. We will share our expertise with other laboratories which will then be able to apply the same methodologies.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We will use several cichlid species and throughout the duration of this project (five years) we plan to describe the function of 15 genes. For this purpose, we will generate 15 groups of fishes that harbour gene modifications (mutations). Each group is technically defined as a strain. There are more than 15 genes affecting colouration but due to time constrains during the next five years we will only be able to tackle the function of this subset. We will use methods that involve in vitro fertilisation (joining eggs and sperm in a tube to obtain a fertilised egg) and injection of cichlid eggs with particles that are able to modify specific gene portions.

To collect eggs and sperm, we need 10 individuals per species and per strain. We plan to collect eggs and sperm from three different species (total of 30 individuals) and from 15 gene deletion strains (150 individuals).

To generate and maintain the different gene deletion strains we require 300 individuals (total of 4690 individuals for the 15 gene deletion strains).

Finally, we will image how colour appears in a subset of juvenile fish, for this purpose we will require 500 individuals.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

What we propose to do to the animals will minimally impact on their health and welfare because we will only modify genes that are involved in different skin colours.

Adult fish will be anaesthetised and we will perform a gentle abdominal massage to collect eggs and sperm. After collection, we will perform in vitro fertilisation or store the sperm for future usage. In vitro fertilisation will originate cichlid embryos that will be maintained in aquaria in our facility.

We will inject fertilised eggs with particles that modify specific gene portions. These modifications should only affect the colour of the fish, but might also result in harmful growth malformations (e.g. fish embryos that do not grow, embryos that lack organs, juvenile fish that swim and behave abnormally, etc.). If the latter occurs affected animals will be humanely killed as soon as the malformation is detected.

Fish showing colour modifications will be imaged throughout time, while the colour pattern is developing. To improve the imaging, individuals will be anesthetised and subjected to a substance that contracts the pigmented cells that make up the colour pattern.

Animals that are not fully recovered at the end of the procedures will be killed humanely.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

To understand the relationship between mutations and differences in colouration, we modify specific genes to assess their function on the colour of juvenile and adult fish. We therefore cannot avoid using animals for the purpose of this project.

Throughout the project, we will implement strategies to replace animal use:

1. For each humanely killed fish we will dissect and store all its tissues (e.g. brain, heart and skin). This tissue archive will be made available to other researchers, which will replace the use of live

animals in their laboratories.

2. We will develop a sperm freezing protocol, which will allow us to store and revive cichlid mutant strains, via *in vitro* fertilisation, replacing the need to keep adult fish with gene deletions in our aquatic facility.

Reduction

Explain how you will assure the use of minimum numbers of animals.

We will strive to use the optimum number of animals and throughout the duration of the project will continuously consider if the benefits of the number of animals used and procedures performed outweigh the potential harm. We will plan, conduct and report our experiments according to the PREPARE and ARRIVE guidelines.

For the egg and sperm collection, animals used will be kept to a minimum required to perform successful *in vitro* fertilisation and sperm and eggs will be frozen and stored to reduce the need to use more individuals.

For the generation and breeding of cichlid genetically altered strains, only individuals with confirmed gene modifications will be used. Gene modifications are confirmed by collecting tissue biopsies (e.g. by removing a very small piece of fin or collecting skin swabs), which are then used for genetic tests. Fish harbouring genetic modifications that are not actively used in experiments will be preserved by sperm freezing which will reduced the number animals used in the facility. If we need to resurrect a line, we will perform *in vitro* fertilisation using previously frozen eggs and sperm.

For the repeated imaging of pigmentation development in cichlids showing colour modifications, animals used will be kept to a minimum required to detect differences in colour pattern formation. Finally, we have amended our licence to changed the maximum age at which we have to cull our GA fish to 60 months, which will reduce the number of fish required to complete the experiments.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The species we proposed to use are large, robust and thrive in an aquaria environment and will withstand the mild regulated procedures with the minimum of stress. In order to continuously minimize animal suffering we will always use up to date technology and refine the housing environment.

Cichlids will be housed in aquaria mimicking their natural environment, which is a sandy area rich in vegetation and hiding stones. Sand, artificial plants, plastic tunnels and clay pots will be used to enrich their aquaria environments. Group housing decreases stress and aggression levels in fish. We will maintain adult fish in high-density groups to reduce aggression, thus avoid unnecessary stress or

injuries from chasing or fighting. Researchers and animal technicians will pay close attention to the fish in every tank, in order to control stress and aggression levels. We will continuously run trials on optimising feeding regimes and control water quality to ensure optimal husbandry.

For the repeated imaging of pigmentation development in cichlids showing colour modifications, imaging will first be performed every five days to limit exposure to the anaesthetic and to the substance that causes pigment cells to contract. We will only shorten the interval between imaging sessions if deemed necessary, i.e. if the colour pattern changes too fast for differences to be detected. Furthermore, the final anaesthetic treatment will be performed under terminal anaesthetic, such that the fish will not have to undergo the stress of anaesthetic recovery before being killed using a Schedule 1 method.