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NON-TECHNICAL SUMMARY

The role of transposable elements in cichlid fish development and evolution

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Transposable elements, Gene silencing, Epigenetics, Adaptive Evolution, East African cichlids

Animal types

Haplochromine cichlid fish of the East African Great Lakes.

Life stages

adult, juvenile, neonate, embryo

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

To understand the role of certain genes, termed transposable elements, in the evolution of East African cichlids.

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.

Why is it important to undertake this work?

It is important to identify the genes underlying the evolution of animals. The knowledge of the genetic basis of evolution is relevant, for example, to predict how animals react and adapt to a fast changing environment. This understanding is most important nowadays given global climate change trends, which are increasingly having a deep negative effect on biodiversity. This is particularly relevant for animals that live in a "closed" environment, from which they cannot migrate out of. Such is the case of fishes specialised to life in lakes.

Transposable elements are genes that exist in most animals. The special feature of these genes is that they can multiply and change their location within cells. As this change in location may damage other genes, yet another set of animal genes is dedicated to the control of the mobility of transposable elements. However, the mobility of transposable elements does not have only negative effects. In fact, some transposable elements may lose their ability to change location and become fixed in one location (termed "domesticated"), by evolving a useful function.

In the proposed research we will use a fascinating group of animal species, the cichlid fish of the East African Great Lakes, to investigate two aspects related to transposable elements:

- 1) the contribution of "domesticated" transposable elements to the evolution of these fishes; and
- 2) the genes that prevent the mobility of transposable elements.

What outputs do you think you will see at the end of this project?

Due to the presence of transposable elements in most animals and their ability to multiply, these genes are responsible for providing new genetic diversity that may, in some cases, be important for evolution. Genetic alterations of transposable elements and the genes that control them is required to understand what their function is in animals.

The results of the proposed work, using genetically altered animals, have the potential to result in several publications reviewed by other scientists. These publications will report findings related to the following main topics:

- 1) Identification of the genes controlling the mobility of transposable elements in cichlid fish, across several relevant parts of the body (including the reproductive organs and the brain), as well as in eggs and sperm.
- 2) Determination of whether the activity of transposable elements is affected in animals created by mating individuals of different species.

3) Identification and characterisation of "domesticated" transposable elements important for the evolution of these fishes.

Furthermore, it is likely that the data generated in our work will be useful to other researchers and lead to collaborative work. Importantly, our data will be deposited in publicly available, free repositories, so that other researchers can download it and use it to answer their own biological questions. This is also a reduction measure, as it should avoid repetition of the same work by other researchers.

These publications and collaborations are direct benefits resulting of this work, which will be likely produced within the timeframe of the license and shortly after. It is also likely that our publications will provide a substantial contribution on the medium-/long-term to biological research.

Who or what will benefit from these outputs, and how?

Our research is of a fundamental nature and will build important knowledge on the "domestication" of transposable elements and on the genes that control transposable elements. This will increase our understanding of nature and of the genes implicated in animal evolution. As transposable elements exist in most animals, our discoveries may be relevant to understand the evolution of other groups of animals, including mammals. As our project touches upon so many fundamental biological aspects, we expect to impact on several fields of the biological sciences, e.g. evolution, molecular biology, and genetics.

Given the fundamental nature of the proposed work, the research output is not likely to produce directly any product or method with commercial potential.

How will you look to maximise the outputs of this work?

I intend to present my results in international and local scientific conferences, or other scientific meetings. I will attend, at least, one major scientific conference per year on topics relevant to my proposed research. These conferences will provide opportunities to communicate my results broadly. Other persons involved in this work will also participate in dissemination efforts.

Our investigations and the data generated are expected to lead to new collaborations with other researchers. Our work will be published in free, public repositories online and in international scientific journals.

The genetically altered animals, or their eggs/sperm, will be openly available and shared with the scientific community, if requested and assuming all legal permissions are in place. Sharing animals worldwide will replace the creation of novel genetically altered animals, and reduce the total number of animals used in animal research.

Species and numbers of animals expected to be used

- Other fish: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.**Explain why you are using these types of animals and your choice of life stages.**

We are using East African cichlids of the African Great Lakes due to their unique and fascinating diversity of body sizes, shapes, diets, and behaviours. Although they vary in these ways, they do not vary much genetically, in fact being genetically very similar. The combination of these features make them, as a whole, a great system to pinpoint the genes underlying cichlid evolution.

It is beneficial for transposable elements to be active and multiply in the reproductive organs, eggs or sperm, or during early development, as this increases their chances of transmission to the next generation. Thus, the activity of transposable elements and of the genes that control them should be studied in the reproductive organs and during early development. In addition, we discovered genes that inhibit transposable element activity in the brain. This raises the interesting possibility that transposable elements are important for brain development and function. These exciting preliminary data justify the use of these parts of the body.

Typically, what will be done to an animal used in your project?

Creation of genetic alterations - fish eggs will be gently held and injected so as not to damage the egg. Immediately after, the eggs will be returned to aquaria. Injection of a batch of 25 eggs takes less than 20 minutes. Injections will be performed to create genetic alterations, meaning the addition or deletion of genetic material. Genetic alterations will be generated in transposable element genes, or genes involved in the control of transposable elements. These alterations will, in principle, not cause lethality.

Confirmation of the genetic alterations - After the creation of genetic alterations by injection of fish eggs, fish will be grown until they reach their juvenile stage. At this point, we will confirm the genetic alterations that we introduced. This will be done either by cutting a little portion of a fin, or by taking a swab from the fish. Swabs will be preferred to fin cutting, as recent data indicates that swabs provide the more refined method. Swabs gently collect skin cells and mucus, in a way that does not damage the scales and skin of the fish. When confirming genetic alterations using a portion of the fin, the amount of fin removed will not compromise swimming or any other aspects of normal fish physiology or behaviour, and taking the sample takes less than a minute. The cichlid fish we are using are large and robust. Therefore, these procedures will be preferentially performed without anaesthesia, as this brings a higher risk of adverse effects.

Isolation of eggs and sperm - Adult fish will be anaesthetised and eggs and sperm will be obtained by applying gentle pressure on the sides of the fish.

What are the expected impacts and/or adverse effects for the animals during your project?

Our proposed procedures will impact the animals' health and welfare minimally and will not lead to long-lasting pain, suffering, or distress. The genetic alterations we propose to introduce are highly unlikely to lead to any malformations or adverse effects. Some eggs may be damaged during the injection procedure, but these, whenever detected, will not be allowed to live past the stage where they are still very small and immature fish, before they start to feed independently. East African cichlids are

large and will withstand well our proposed procedures. These procedures will be performed in the least amount of time possible and are not expected to lead to any long-term consequences or distress. After any procedure, animals will be monitored for an adequate amount of time to ensure recovery.

Animals are expected to remain healthy and normal and, should this not be the case, they will be humanely killed immediately to prevent any pain, suffering, or distress.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Fish: Mild severity: 100%.

What will happen to animals at the end of this project?

- Killed
- Used in other projects
- Kept alive

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Transposable elements are important genes for animal evolution. However, their roles in the evolution of East African cichlids remain unknown. We aim to understand if and how transposable elements influenced the evolution of diverse colours, shapes, behaviours, and dietary adaptations in these fishes. To conduct this investigation we need to generate cichlid fishes with genetic alterations.

Which non-animal alternatives did you consider for use in this project?

We have considered computer simulations and cultures of cells.

Why were they not suitable?

At the moment there are no alternative resources capable of recreating the natural development of fish. Likewise, eggs and sperm are created and maintained in a unique and complex natural environment impossible to replicate in the laboratory. In order to fully understand animal evolution, we need to understand the role of transposable elements. To do so, we need to see how live animals with specific genetic alterations look and behave in a natural context. Other alternative systems, such as cell culture, or computer simulations, are not able to recreate the complex interactions that occur during

development, between neighbouring cells and tissues. These interactions are most important for proper animal development. Thus, our aim cannot be accomplished without using animals.

We will use methods to collect mature sperm and eggs of cichlids, effectively bypassing the need to kill adult animals for the dissection of their reproductive organs. Eggs and sperm will be used as described, or frozen after their isolation. As these frozen eggs and sperm can be thawed and combined together in the laboratory to create new animals, their isolation will allow for the long-term conservation of our animals. Generation of animals in the laboratory will replace the continuous breeding of specific cichlids, when not in use.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We plan to produce animals with 3 distinct types of genetic alterations (**maximum of 3000 animals**):

- 1) We plan to produce up to 5 lines of animals with added genetic material. For their breeding, we expect to use a maximum of **1000 animals** (5x200).
- 2) In addition, we will alter or delete genetic material of up to 5 genes required for preventing the activity of transposable elements. For their breeding we will need a maximum of **1000 animals** (5x200).
- 3) We plan to alter or delete genetic material of up to 5 transposable elements in cichlids. We expect to need a maximum of **1000 animals** (5x200) to breed these animals for the duration of the project.

Lastly, we aim to collect eggs and sperm from a maximum of **200 individuals** (200 protocol 2).

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Genetic alterations will be performed on a carefully chosen set of genes. At the beginning of this research programme, we used computational methods and available data to confirm that the candidate genes are active in the tissues of interest. In this programme of work, validation of our candidates will be performed using procedures not regulated by law. This careful consideration and preliminary analysis ensures our set of candidate genes are most relevant to the biological questions we aim to answer.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Throughout the duration of the project, we will use the minimum number of animals required to acquire satisfactory data. Experiments will be designed, conducted, and reported according to published guidelines (namely the PREPARE and ARRIVE guidelines).

Whenever an animal is culled, a variety of tissues will be extracted and frozen. As other colleagues may have an interest in tissues not necessarily relevant for the purpose of this project (e.g. scales, muscle, liver), tissue sharing will avoid culling of additional animals. Similarly, sharing data obtained from dissected tissues with colleagues will avoid culling of additional animals for repetition of these experiments.

Frozen eggs and sperm will ensure the long-term maintenance of fish lines. This practice will reduce the number of individuals bred, when they are not required, and will prevent duplication of experiments (i.e. re-generating animals with particular genetic alterations).

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

I will use East African cichlid fish for the proposed work plan. As these fish species are large and robust, they can be effectively bred and grown in an aquaria environment. Moreover, these animals can tolerate the mild regulated procedures we propose to perform with the minimum amount of stress and pain.

The egg and sperm collection, the creation of genetic alterations and their confirmation will be conducted using current best practices and technology. These procedures are expected to cause little pain, suffering, and distress to the animals. Genetic alterations will be created by injection of eggs, an immature life stage before the animals feed independently. None of our experiments will require animals to live in isolation.

Why can't you use animals that are less sentient?

The work proposed here takes advantage of the extreme diversity of shapes, sizes, colours, diets, and behaviours of East African cichlids, in spite of very close genetic similarity. The diversity of these cichlid fishes is unique amongst vertebrates and even amongst the entire animal kingdom. Therefore, other less sentient, or invertebrate, animals will not allow us to answer the same biological questions.

I will use whenever possible tissues obtained from immature life stages, i.e. from early development, before the animals reach the independent feeding stage. Tissue dissection and extraction, besides the methods to confirm the genetic alterations, will be exclusively performed in dead animals.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will incorporate new recommendations on animal welfare, as soon as possible after their publication.

Cichlid housing will be continuously improved to faithfully mimic the sandy areas rich in vegetation and hiding places characteristic of their natural environment. This will be achieved by introducing sand, artificial plants, clay pots, and plastic tunnels in the aquaria. Fish will be housed in groups, in order to decrease stress and aggression levels. Manifestations of aggression, including chasing, fighting, and injuries will be monitored daily by the scientific and animal care staff. These aggressive behaviours are normal to these species and not a product of captivity. If required, fish will be temporarily separated and groups redesigned to avoid further injury and aggression. Continuous monitoring of water parameters, food regimen, and breeding will ensure the wellbeing of the animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I will follow the best practice guidelines available at www.nc3rs.org.uk. Experiments will be planned, documented, and reported according to published guidelines (namely the PREPARE and ARRIVE guidelines).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I will follow the website of the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs, available at www.nc3rs.org.uk), and stay up-to-date with new information and new resources that become available. To achieve this, I have signed-up to the NC3Rs e-newsletter. In addition, named persons at my establishment (i.e. the Named Animal Care and Welfare Officer, Named Veterinary Surgeon, and Named Information Officer) will inform us of relevant new information and resources, and will advise, together with the animal care staff, on the best ways of implementation.