Genetic control of Grey Squirrel populations in the UK through gene editing: Directed Inheritance Gender Bias (DIGB)

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1. Executive Summary

- The grey squirrel is one of the best-known invasive non-native species in the UK.
- Invasive non-native grey squirrels cost the UK in the order of £40m per year, in tree damage alone.
- Grey squirrel numbers are controlled to protect red squirrel populations and prevent them from damaging and killing trees, both broadleaved and conifer.
- Current squirrel control measures are considered by many to be inhumane; they are all costly and labour intensive; not specific for grey squirrels; and at best only transiently control grey squirrel numbers.
- The UK Squirrel Accord is supporting the development of an immuno-contraceptive approach to control grey squirrel numbers; progress is being made but this strategy still suffers from being costly and labour intensive to deploy while difficult to specifically target grey squirrels and will only reduce numbers. (likely collateral effect on other small animals).
- We propose a genetic solution to eradicate grey squirrel populations locally to contribute to maximising the benefit from the national drive to plant 30,000 hectares of trees per year.
- Directed Inheritance Gender Bias (DIGB) relies on genetic engineering technology; specifically, DIGB relies on gene drive, a proven innovative application of genome editing and GMO technology.
- DIGB offers a genetic alternative 'contraception' by skewing the sex ratio within the target population leading to a population crash.
- For less than the annual cost burden of grey squirrels, DIGB could be developed for £10m.
- DIGB could be used to humanely eradicate the grey Squirrel from regions within 20 years.
- DIGB technology could be used against other invasive non-native species.

2. Financial Commitment

DIGB is currently in the research phase at The Roslin Institute, University of Edinburgh). It would take 5 years of development to have a product ready for deployment. It would then take 10-15 to achieve a major step towards eradication.

Year 1-3: Research (development of gene drive tools and delivery strategies) = £3m

Year 1-5: Establishing assisted reproductive technology grey Squirrel facility = £5m

- **By 2026: Product (Gene Drive Grey Squirrels) ready for field testing = £8m**

Year 5-8: Field trials for DIGB = £2m

- **By 2029: Product (Gene Drive Grey Squirrels) ready for deployment = £2m**

Total development costs = £10m

Year 8-20: Deployment in UK forests = £10-20m

- from 2035: Control and targeted eradication of grey squirrel population numbers achieved

3. Overview of Proposal

Two phases are proposed: the first, research to field validated product (gene drive squirrels); the second, deployment of gene drive squirrels to UK forests.

Phase 1 <u>research</u> to field validated <u>product</u> overview:

(i) Production of gene drive reagents (year 1-3)
- gene drive reagents generated
- gene drive reagents validated in cell culture
- conformation of target genetic loci in experimental mice

(ii) Strategizing deployment routes (year 1-3)
- in silico modelling of deployment strategies *
- to define field release parameters
- to inform public consultation activity

(iii) Establish required infrastructure (year 1-5)
- establish Squirrel assisted reproductive technology experimental facility (ARTEF)
- secure appropriate regulatory approvals
- development of gene drive delivery approaches (to produce founder animals)
- produce test gene drive squirrels (in containment)
- validate gene drive in ARTEF (Contained Use study)

[Development Milestone 1: proof-in-practice of gene drive in Squirrels]

(iv) Field Trials (year 5-8)
- Identification of release sites
- secure appropriate regulatory approval for field trials (Deliberate Release)
- perform field trials in selected forests
- field trial informed refinement of deployment strategy
- further public consultation activity

[Development Milestone 2: field trial data]

Phase 2 product <u>deployment</u> overview:

(v) Gene drive squirrel release (year 8-20)
- phased release of gene drive squirrels
- monitoring of release gene drive animals in forests
- refine modelling of gene drive distribution and impact to reflect real deployment data

* Deployment Parameters. We have performed an initial modelling study (Nicky Faber et al, publication in press) of a conservative gene drive strategy indicating that release of 100 squirrels in a region harbouring 3,000 squirrels could achieve eradication over a 20 year period. Further modelling will enable the deployment parameters to be refined to identify optimal release numbers, reduce time to eradication predictions, etc.

4. Background

(i) Squirrels

Grey squirrels (*Sciurus carolinensis*) are an invasive non-native animal in the. They cost the forestry industry £40m per year. In addition to providing rural employment and income, forestry is the basis for some of the traditional and most sensitive ecosystems of the UK.

Grey squirrels are rapidly pushing our native red Squirrel (*Sciurus vulgaris*) to extinction in the UK. The grey Squirrel is both more aggressive and out competes the red Squirrel for food and living space. More devastatingly, the grey squirrel is a carrier of squirrel pox which is lethal to the red Squirrel. There is no vaccine and no treatment for squirrel pox available. It is predicted that without effective conservation measures, the red squirrel could be lost from the UK by 2030.

(ii) DIGB (using Gene Drive technology)

Advanced genetic technologies, including genome editing and GM now offer an innovative strategy – called gene drive – to control the animal populations. As each species has a unique genome (their DNA), these reagents can be tailored to work only on the target species. Thus, the gene drives will only work in the target species (e.g. work in the grey squirrel but not in the red squirrel, thus offering a species target solution).

There is considerable current activity developing gene drive strategies to control mosquito populations and mitigate disease transmission by these insects.

Animals have about 20,000 genes in their genome. In all but germ cells (sperm and eggs) animals carry two copies of each gene (these copies are termed “alleles”) in each of the cells that make up the animal: they inherit one copy from their father and one copy from their mother. Since both the father and mother have two copies themselves, there is a 50% chance of inheriting a given copy from a given parent. Therefore, if one parent has a ‘desirable’ copy and the other has no desirable copies, only 25% of the offspring will carry the desirable copy (50% of 50%).

In one type of gene drive, the single desirable copy (the introduced GM) is driven through a population such that all offspring carry it. That animal will pass the GM to all of their offspring and so on. This technology has only recently become possible through the development of genome editing tools. DIGB is a composite of GM and genome editing technology (and requires appropriate regulatory processes).

Gene drive technology can be used to distort the sex ratio of a population – which is usually about 50:50 females to males. In this scenario a gene drive is established in the target animal (i.e. grey squirrel) that causes female sterility. Over time this will lead to a preponderance of males (the opposite effect can also be achieved; male sterility leading to a preponderance of females); this skewing of sex ratio within the target population will lead to a population crash due to the absence of female animals.

(iii) Broad application to control invasive non-native species and pests

Invasive non-native species not only challenge the survival of our rarest species but damage some of our most sensitive ecosystems, costing the UK economy more than £1.7 billion per year. DIGB as currently considered is designed to control grey squirrel population numbers. Once established and proven, DIGB could be adapted for any invasive non-native or animal pest species.