Gene Drive – a game-changing technology for grey squirrel control

Stef Kaiser speaks to Bruce Whitelaw, Prof of Animal Biotechnology at Roslin Institute.

Grey squirrels *(Sciurus carolinensis)* are an invasive, non-native animal in the UK and are a threat to the health of woodland ecosystems. Tree damage caused by squirrels costs the forestry industry £40m per year.

In addition, grey squirrels are rapidly pushing the UK's native red squirrel *(Sciurus vulgaris)* to extinction. It is predicted that without effective conservation measures, the red squirrel could be lost from the UK by 2030.

The UK Squirrel Accord is backing the development of an immuno-contraceptive approach to control grey squirrel numbers. In parallel, the Roslin Institute in Edinburgh, in collaboration with the European Squirrel Initiative, Edinburgh Innovations and the Biotechnology and Biological Sciences Council, proposes an innovative genetic control strategy to complement existing methods. The suggested technology, 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. It could be used to humanely eradicate the grey squirrel from regions within 20 years and could be applied for the control of other invasive, non-native species.

GENE DRIVE TO CONTROL GREY SQUIRREL POPULATIONS

- Drive infertility through the target population
- Genetic equivalent to immunocontraception
- Can be used in parallel to other control strategies
- Technology applicable to other pest species
- Humane (no animals killed)
- Specific for grey squirrel (no collateral)
- Possibility to limit gene drive to a number of generations.
- 5 + 3 yrs R&D (£10m)
- Then impact in 15-20 years
- Now seeking £10m funding for R&D phase

THE TECHNOLOGY

using Gene Drive for directed inheritance gender bias

Gene drive is the use of a genetic engineering tools to 'drive' a desired genetic trait through a population by increasing the probability that the trait will be transmitted to future generations (figure 1). It is currently being developed for use in insects, and the Roslin Institute proposes that the technology could be tailored to cause female infertility and be used to control grey squirrel numbers in our woods and forests.

Normally, what happens with inheritance is that both females and males carry two copies of the same gene (the copies are called 'alleles') but each parent only passes on one copy of the gene to offspring. Unless there is a selection pressure for a given allele, half of the first generation of offspring will carry the gene of interest, half will not and so on through generations (figure 1, left side).

Gene drive changes this inheritance pattern for the gene of interest with the aim that, theoretically, at some point 100% of the population will carry the gene.

The gene drive technique involves adding, deleting, disrupting, or modifying genes by using 'transgenes'. For gene drive to work, a transgene is inserted in

one allele of the gene (figure 2, left).

"As soon as we've done that, the introduced gene drive activates and copies itself into the other copy of the gene. Now, this animal has two copies of the desired transgene and, rather than 50%, all gametes (sperm or egg cells) will carry the gene drive. When a gene drive animal mates with a non-gene drive partner and fertilisation happens, the same copying happens again, and the offspring, once more, will have two copies of the desired gene. This way, the desired trait 'drives itself' through the population", explains Prof Whitelaw.

Gene drive can be used to 'drive' any trait, such as disease resistance or, in this case, infertility through a target population. In the case of the proposed project, the gene drive will be used to drive female infertility, and over time, this will lead to a preponderance of males. Eventually, the population will crash due to an absence of female animals.

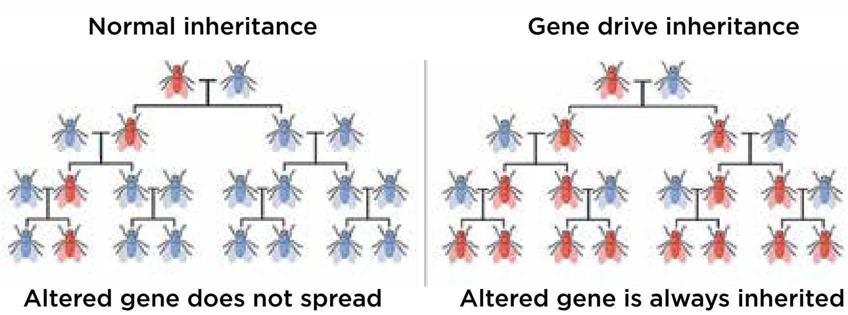

Figure 1

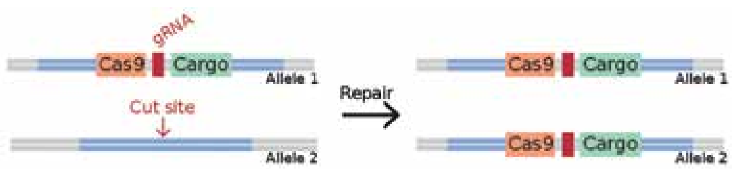

Figure 2

CREATION AND DEPLOYMENT

of genetically modified squirrels

This project is at an early stage an no animals have been produced. Looking forward, gene drive grey squirrels could be engineered. Such animals, who will be males, would be released into the target woodland. After natural mating, those males will pass their gene drive on to their offspring – and produce fertile males and infertile female.

Thanks to funding from the ESI, researchers have performed an initial simulation study *(Nicky Faber et al, 2021.Scientific Reports 11, 3719)* of a conservative gene drive strategy indicating that the release of 100 squirrels in a region harbouring 3000 squirrels could achieve eradication over a 20-year period (figure 3). This assumes a one-year breeding cycle. Further modelling will enable the deployment parameters to be refined to identify optimal release number and, reduce time to eradication predictions.

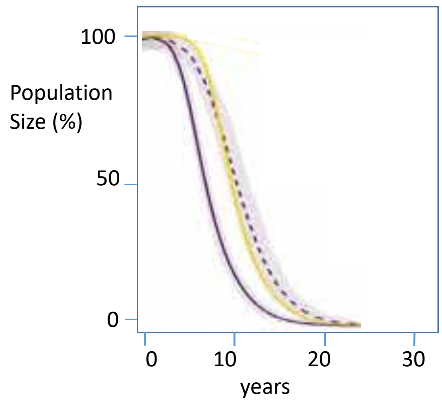

Figure 3

The team at Roslin will closely work with ecologists to model the impact of reducing grey squirrels in a particular ecosystem, and red squirrels filling the ecological niche.

Although there is considerable current activity developing gene drive strategies to control mosquito populations and mitigate disease transmission by these insects, using gene drive in squirrels would be pioneering, it hasn't been done before. There is no gene drive work in the world beyond experimental in mice, which was conducted in the US – with very valuable lessons learned about limitations of the technology.

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ASSESSING AND MITIGATING RISKS

When it comes to genetic engineering, a main concern for both the public and funding bodies will be the potential existence of unintended or unpredictable consequences on other species or the ecosystems as a whole, when genetically modified organisms are introduced into the wild. Currently, regulations for approval of GM projects are very strict, with a strong bias towards the principle of precaution. Equally, there is a consequence of not trying new control approaches to a pest animal which continues to expand its footprint in the UK.

Using the DIGB technology for grey squirrel control would come under Defra's GMO regulations – with the outome of their recent consulation on genetic technologies imminent.

Is there a risk of red squirrels or other species becoming infertile?
The first barrier to contamination of other species is the so-called 'species barrier'. The gene drive can only be transmitted to a animals that a grey squirrel could mate with. There are situations where certain similar species can inter-mate but usually the offspring will be sterile.

Beyond that, the gene drive will be tailor-designed to work only on DNA sequences that are exclusive to grey squirrels, or even a specific grey squirrel population. These 'personal sequences' are a common genetic denominator of a certain group of individuals (species, breeds or even families) that is different to everyone else. To illustrate this, let's say we wanted to target an isolated population of grey squirrels in Aberdeen. It is possible that these squirrels have slightly diverged from other populations and therefore have 'personal sequences'. It would be possible to design a gene drive that just works in this local population in Aberdeen. The likelihood of this gene to be introduced into other populations or even species would be in effect zero.

Is there a risk of eradicating the species as a whole, even in its native habitat, for example, by accidental return of individuals to their original habitat?
This risk does exist. However, there are tools to design strategies that limit the number of generations for the gene drive; this means that we can 'dial-in' an end point where the infertility trait doesn't get inherited anymore. This can be imagined a bit like 'planned obsolence' in electronic devices, or commercial seeds only working for a specific number of years chosen by the manufacturer.

An example for such a strategy is the 'Daisy Chain': within the transgene, there are in this case four gene drive sequences, with one targeting the next until the last sequence has no target and the gene drive stops. This strategy has been proven in mosquitoes under laboratory conditions.

Several parameters allow researchers to create a safe and bespoke grey squirrel control strategy that reconciles efficiency and speed of eradication with maximum risk mitigation. The choice of the genetic target sequence (to ensure specificity), the number, location and timing of animals released, and the number of generations the gene drive should work.

RESEARCH TIMELINE AND COST

A outline timeline to develop the DIBG project would be:

- **Years 1-3:** refinement and validation of gene drive technology and reagents
- **Years 3-5:** production of gene drive grey squirrels (in containment)
- **Years 5-8:** contained use field trials
- **Years 8-25:** deployment in our woods and forest

The project cost is estimated at £10 million, and the research consortium is currently looking for funding to start the research phase.

QUESTIONS FROM THE CONFOR COMMUNITY

Q What is the desired outcome – totally remove species or remove numbers?
Conceptually, we can either reduce the population or aim for a complete eradication at a local or regional level. Grey squirrels are an alien species that has negative impact on the environment; ESI's long-term aim is to see eradication of the species and for red squirrels to take their space.

Q Do you anticipate public opinion as a major challenge for funding and implementing this technology?
The public will have reservations against any kind of animal control. However, explaining the bigger picture and the reasoning behind control of invasive species usually has a very positive effect on public opinion, says Graham Taylor of the ESI.

The main aspects people are concerned with, in particular in the case of GM animals, is animal welfare and sustainability. In this sense, Prof Whitelaw believes that using this gene drive strategy to control grey squirrels is exactly what the public want –"it's humane and it's safe for the environment".

In Australia, scientists are looking into utilising the technique as a way of controlling rodents. Interestingly, it is PETA, an NGOs who you might not suspect of being supportive of biotech, who are coming out in support of this technology.

Q What is the likelihood of mutations occurring that would render the gene drive mechanism ineffective in 10 or 20 years?
Yes, mutations are a natural process and would limit the gene drive. There would be a natural selection favouring those mutations, once they appeared. As the model will be based on the gene drive working for a limited number of generations anyways, the effect of spontaneously occurring mutations on the efficiency of our control method should be negligible. In addition, sequential gene drives could be deployed to mitigate this.

Q How will trial areas be selected?
The initial trials would be conducted in containment facilities and after that, it could be trialled in specific forests. We do get a lot of interest from landowners who offer their forest for trials. The ideal site for an open trial would be an island with only grey squirrels – but that is wishful thinking, of course. But we could use 'island populations' of grey squirrels on the mainland. No GM squirrels have been produced yet, and we are still far away from the open trial phase.

Q What are the constraints of the technology?
The biological constraints are that no-one has ever genetically engineered a squirrel and we'd have to set up the practical capability to do that. In Roslin, we use similar strategies for mice, rats, chickens, pigs, sheep and cattle so we are confident that we can adapt the methods to squirrels.

Although gene drive has worked in insects and experimentally in mice, we haven't got any practical experience with the proposed technology working in squirrels. We might have to try a few target sequences and gene drives to figure out which one is the most effective.

We are still in the development phase, but we don't think any of the challenges are insurmountable. The insect research community will provide useful information that we can use for our R&D.