ISBR Workshop: "Gene editing and gene drives for managing unwanted vertebrates – current status and biosafety considerations"
Tuesday, April 2nd, 2019; 14.00-17.25; Tarragona, Spain **http://www.palautarragona.com/en/**
Room Galba

Co-organizers: Dr. Allison Snow and Dr. Tim Harvey-Samuel
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Gene editing and gene drives have the potential to alter or suppress populations of unwanted vertebrates such as mice, carp, and feral cats. For example, gene editing has been proposed for blocking Lyme disease in white-footed mice, while gene drives have been proposed for eliminating invasive house mice, rats, and feral cats that threaten endangered species on islands. However, the time frame and feasibility of developing such applications is not clear. The goal of this workshop is to provide updates on scientific progress related to the first applications of these techniques in wild vertebrates. Presentations by six invited speakers will be followed by a panel discussion, with time allocated for questions from the audience. For each study species, speakers will review long-term project goals and preliminary research findings relevant to feasibility and biosafety considerations. Research updates could cover studies to identify sterility genes; gene drives for altered phenotypes; methods for avoiding mutational breakdown of introduced traits; biological confinement strategies; population genetics and gene flow; hybridization, mating behavior, and dispersal; rearing methods for wild species; and data needed for ecological risk assessments. Speakers will identify key research gaps and regulatory approvals that must be addressed during the development of each proposed application. Following these talks, a panel of experts will summarize the status and prospects for gene drive applications. Although public debate is essential for applications of genetically engineered vertebrates, ethical, social, and political issues will not be addressed here due to time constraints. A better understanding of the first proposed applications and anticipated time frames for possible release will be useful to social scientists, regulatory agencies, funding agencies, science writers, NGOs, public stakeholders, and others. Gene drives represent an emerging technology with many imagined applications, consistent with the Symposium theme, "New Horizons in Biotechnology: Risk Analysis for a Sustainable Future."

Schedule -

14.00 Context setting by workshop co-chairs Allison Snow and Tim Harvey-Samuel
14.10 Allison Snow, Ohio State Univ. – "Ecological context for releasing Lyme-resistant white-footed mice: a case study of gene editing"
14.30 Owain Edwards, CSIRO, AU – "Determining the feasibility of gene drives for feral cat control in Australia"
14.50 Gus McFarlane, Roslin Institute, Univ. of Edinburgh – "A CRISPR split-drive targeting female reproduction in mice"
15.10 Paul Thomas, Univ. of Adelaide, AU – "Safe development of CRISPR gene drives for invasive rodent population suppression"
15.30 Coffee break (symposium-wide)
15.50 Michael Smanski, Univ. of Minnesota – "Engineering incompatibility and applications for controlling invasive fish populations"
16.10 Keith Hayes, CSIRO, AU - "Principles of probabilistic risk assessment for genetic biocontrol"
16.30 – 17.25 PANEL DISCUSSION - Tim Harvey-Samuel (Pirbright Inst., UK), Owain Edwards (CSIRO, AU), Neil Gemmel (Univ. Otago, NZ), Keith Hayes (CSIRO, UA)

Each panel member will give a 5-10 min. summary of these topics, followed time for questions from the audience:

1) In your opinion, what are some examples of vertebrate species and traits that may be among the first gene drive applications, and why?
2) What is your view of the potential scientific challenges of developing gene drives for these examples?

ABSTRACTS

1) Ecological context for the proposed release of Lyme-resistant, white-footed mice: a case study of gene editing

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Genetic engineering of wild populations has been proposed as a way to reduce human diseases by altering pathogens' hosts. For example, gene editing may be used to create white-footed mice (*Peromyscus leucopus*) that are resistant to the Lyme spirochete vectored by blacklegged ticks (*Ixodes scapularis*) in the USA. Towards this goal, Kevin Esvelt and colleagues began working with residents of Nantucket and Martha's Vineyard islands, Massachusetts, in 2016 to discuss genetically engineering local white-footed mice, which are the primary reservoir host for Lyme. As proposed, if field trials with gene-edited mice on small islands show that the incidence of spirochetes in mice is reduced substantially, the project would scale up to Nantucket and Martha's Vineyard and possibly the mainland (perhaps by adding a localized gene drive), pending approvals from relevant constituents. White-footed mice are abundant in forest habitats and they play a major role in natural predator-prey-herbivore interactions. To evaluate possible hazards of releasing GE mice, detailed information is needed about the inserted genetic elements, the efficacy of these genetic alterations in conferring resistance, and how the introduced GE mice differ from wild mice with regard to their genetic diversity, behavior, survival, and reproduction. In addition to effects associated with engineered resistance and any founder effects from lab-reared populations, it is possible that insertion of the resistance cassette(s) would have unanticipated pleiotropic effects on mouse phenotypes. A key question is whether the GE mice will have lower or greater fitness than their non-GE counterparts. Fitness experiments carried out under semi-natural conditions can offer insights about such effects, although the full range of such outcomes may not be evident in small-scale, short-term studies. Ideally, the proposed Lyme-resistant, white-footed mice would be very similar to local white-footed mice in their genetic diversity, physiological ecology, fitness, and ecological interactions in local habitats.

2) Determining the feasibility of gene drives for feral cat control in Australia

Owain Edwards[1], **Tanja Strive**[2], **Tim Allard**[3], **Michael Smith**[3], **Karl Campbell**[4], **Andy Sheppard**[2]
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CRISPR-Cas9 genome editing has opened up a new frontier of exploring genetic pest control technologies including gene drives, which can be used to force deleterious traits through target pest populations. Gene drives are delivered and spread through sexual reproduction, which makes population eradication theoretically possible from a small number of released gene drive-carrying individuals. This powerful technology could form part of a fresh new approach to control intractable yet highly damaging invasive species including the feral cat, which in Australia is a key driver of native species extinctions. Indeed, feral cats are estimated to kill over a million birds each day across Australia, and are responsible for 30 endemic mammal extinctions in Australia in the last 200 years. To date, gene drives have been demonstrated effective only in insect and yeast models, and only under controlled laboratory conditions. The GBIRd (Genetic Biocontrol of Invasive Rodents) consortium are developing a gene drive technology to deploy against invasive mice on islands. Once developed, optimised, and proven safe and effective in mice, this technology should be adaptable to target other invasive mammals, including feral cats. In the interim, we are focused on improving our

understanding of feral cat biology, ecology, genetics and behaviour in order to determine whether a gene drive would spread effectively within and between feral cat populations, and if so how long it would take to reach and control feral cat populations across Australia. Understanding genetic diversity, population dynamics, dispersal patterns, mating strategies, etc. is essential to inform release strategies, and any genetic control strategy against cats will need to take into account their territorial nature. We are also engaging with relevant stakeholders and communities across Australia to determine whether, and under what conditions, the Australian public may accept the use of gene drives for feral cat control.

3) A CRISPR-Cas9 split drive targeting female reproduction in mice

Gus McFarlane[1], Bruce Whitelaw[1] and Simon Lillico[1]
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Invasive pests impact the environment, economy and society. Current control methods are costly and largely inadequate, and they often lead to unwanted suffering in target and non-target species. Gene drives that enable super-mendelian inheritance of a transgene may offer a more cost-effective, humane and species-specific alternative than current methods. We set out to develop and test a safe-guarded gene drive system, known as a split drive, that could spread female infertility through a laboratory-contained mouse population. Using mouse embryonic stem cell technology, we developed a Cas9 split drive system which disrupts an essential female fertility gene and confers a recessive female-infertility phenotype. Split drive harbouring embryonic stem cells were developed using plasmid donor-DNA and a combination of Cas9 ribonucleic protein and plasmid-based Cas12a endonuclease. Engineered cells were validated using digital PCR and traditional Southern blotting techniques. A breeding population of split drive mice is being established to study the transmission frequency and phenotypic impact of the drive system in a model mouse population. The findings could help guide the development of safe gene drive systems for vertebrate pest management.

3) Safe development of CRISPR gene drives for invasive rodent population suppression

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Invasive mammalian pests including mice and rats cause significant environmental damage and loss of agricultural productivity. Landscape-scale rodent control relies on widespread distribution of toxic bait and is relatively expensive, labour-intensive and inhumane. Recent advances in genome editing technology suggest that CRISPR-Cas9 gene drives could be used as an alternative, humane strategy for invasive rodent population control. A CRISPR-Cas9 gene drive is a genetic construct that promotes its own inheritance through self-replication and can therefore spread through a given population. Our recent in silico modelling indicates that CRISPR-Cas9 gene drives that induce female sterility or embryonic lethality have potential for eradication of rodents on islands. Our modelling also suggests that a Y chromosome "shredding" drive could be used for invasive rodent population suppression. However, despite their potential, CRISPR-Cas9 gene drives have only been developed in a small number of species including flies, mosquitoes and yeast. Our goal is to develop efficient mouse CRIPSR-Cas9 gene drive technology, incorporating stringent safeguards against unintentional release. Using a ubiquitous Cas9-expressing strain, we have shown that gene drive activation in mouse zygotes promotes generation of indel mutations and not self-replication. We are also developing a

proof-of-concept "germline-active" gene drive using a similar strategy to that employed in insects. We anticipate these experiments will provide an important step towards development of new tools for population suppression of invasive rodent pests.

4) Engineering genetic incompatibility and applications for controlling invasive fish populations

Siba Das, Samuel Erickson, Przemek Bajer, Maciej Maselko, **Michael Smanski**
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We introduce a novel approach to engineer a genetic barrier to sexual reproduction between otherwise compatible populations. Programmable transcription factors drive lethal gene expression in hybrid offspring following undesired mating events. In this talk, I describe the technology, demonstrate a proof-of-concept in yeast, and share recent progress in translating the approach to fish with applications for invasive species control.

5) Principles of probabilistic risk assessment for genetic biocontrol

Keith R Hayes[1]
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National regulations that mandate environmental risk assessments for the contained use and release of Living Modified Organisms (LMOs) typically stipulate the use of qualitative methods. The advent of gene drives, however, have led some agencies to question the adequacy of qualitative risk assessments methods, and The United States National Academies of Science Engineering and Medicine (NASEM) and the Australain Academy of Sciences (AAS) have recently recommended quantitative probabilistic risk assessments in this context. This presentation provides an overview of the principles of probabilistic risk assessment for genetic biocontrol, whilst briefly highlighting a range of methods that enable these principles to be applied. The presentation will draw on examples of hazard analysis and risk assessments completed by the CSIRO DEERA team for real (malaria vector control) and hypothetical (eradication of non-native populations of mice and carp) situations.

PANEL DISCUSSION - **Tim Harvey-Samuel** (Pirbright Inst., UK), **Owain Edwards** (CSIRO, AU), **Neil Gemmel** (Univ. Otago, NZ), **Keith Hayes** (CSIRO, UA)

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