Future Rodent Control Technologies

Safe assessment of CRISPR-Cas9 gene drive strategies in mice

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Invasive vertebrate pests including house mice cause significant environmental damage and loss of agricultural productivity. Current control and eradication methods have limited efficacy. A possible solution to this problem is the genetic modification of entire populations to suppress pest numbers. A CRISPR-Cas9 gene drive is a genetic construct that promotes its own inheritance 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 significant potential for eradication of rodents on islands. However, to date CRISPR-Cas9 gene drives have only been developed in a small number of species including flies, mosquitoes and yeast. Our goal is to develop an efficient mouse CRIPSR-Cas9 gene drive, 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 "germline-active" gene drive using a similar strategy to the successful gene drive experiments in insects. This is the first attempt at developing CRISPR-Cas9 gene drive technology in rodents and provides an important step towards assessing their potential for population suppression of invasive mice.

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Global trends in the development of rodenticides and new approaches

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The history of research discoveries in rodenticide development, their pros and cons as well as current and future-focused research are explored. Research endeavouring to retain essential tools, develop new toxins and delivery systems and explore non-lethal control options has advanced. Lessons learned from the research, registration and technical challenges associated with advancing new technologies are presented. There are exciting opportunities for transformational change based on the integration of existing and new tools, such as advances in wireless technology for species recognition, new self-resetting traps, drones, species-specific toxin-delivery systems enhanced with advanced lures and new toxins which can increasingly combine "low-residue" characteristics with selectivity (e.g norbormide) and humaneness. Putting our efforts into 'silver bullet' technologies for small mammal pest control is the wrong approach to biodiversity conservation. Research and development should focus on blending the practical skill of pest control practitioners with emerging technologies for transformational change, as well as novel fundamental research into biocontrol and new avenues.

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RNAi as the next generation tool for the control of rodent populations

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Rodents cause devastating damage to both agriculture and ecosystems worldwide. Invasive rodents are commonly found on islands, historically free of these animals, and have enormous negative impacts on both native plant and animal species. Rodents are exceptionally well adapted to their environments and therefore, quite challenging to control. Current control strategies often include large scale applications of toxicants, which have potential adverse effects on non-target wildlife. Therefore, the development of new species specific rodenticides would be a valuable advancement in the effort to control these pest species, especially for island eradications. To that end, we are investigating the use of RNA interference, RNAi, as a novel way to control rodent species. RNAi is a new technology that has shown much promise as both a therapeutic for human diseases and in the efforts to control insects and plant diseases. In essence, RNAi is a gene-silencing technology in which small, specifically designed sequences of RNA are introduced into cells and induce the degradation of sequences of RNA encoding a target gene of interest. This degradation of RNA means that the protein for which the RNA was coding is no longer synthesized. By inhibiting protein synthesis, RNAi enables researchers to selectively alter cell function in both normal and disease states. By screening the rodent genome, selecting genes of interest, and comparing the sequences of these genes to non-target species, we are able to choose genes that are present in the pest rodent species and not in the non-target species. Therefore, if non-target species consume the RNAi they will not be effected. The use of RNAi as a method to control pest rodents shows promise because of its species specificity and low non-target impact.

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Opportunities for using novel genetic control tools for the humane control of overabundant vertebrate pest populations

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Novel revolutionary genetic technologies have recently been developed that can force modified genetic traits into an animal population, defying the constraints of normal Mendelian inheritance. A highly specific gene editing system drives the duplication of a specific gene cassette between paternal and maternal chromosomes so that the trait is inherited by all offspring. There is currently very active global debate about the potential applications of this exciting new technology, including novel strategies to humanely control overabundant invasive pest animal populations. Delivered and spread through sexual reproduction the potential of this powerful new technology is unprecedented, making pest eradication theoretically feasible. At present, such technologies have only been shown to function in insects, but work in mice as a mammalian model system is currently underway. Much of the international debate focuses on the risks that might be posed by the technology, how they can be mitigated, how they should be regulated and even whether they can be used at all. The ability to safely control any putative genetic control approaches is paramount, to ensure protection of the target animal in its native distribution range. For any experimental research into genetic control tools it is essential to understand the risks, potential ecological and social implications, to develop physical, genetic and ecological containment measures as well as robust regulatory pathways. In Australia, a world leader in the biological control of invasive species, extensive consultations are currently bringing together key stakeholders. These groups cover a broad range of interests and include scientists from key disciplines, government regulators and public representatives. It is critical to ensure a transparent and informed debate from the outset, responsible conduct of science, and to identify key pathways and barriers to adoption of any putative genetic control tools.

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Evaluation of selected pesticidal plant extracts for maize (*Zea mays* L.) protection against *Mastomys natalensis* (Smith, 1834) in Tanzania

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The Multimammate rat (*Mastomys natalensis* Smith 1884) is a serious rodent pest in Tanzania, which causes damage to maize crops after sowing and during germination resulting into crop loss. In an attempt to reduce the damage, farmers use rodenticides for rodent management. To reduce rodenticides use, an alternative method was sought. The study aimed to investigate ten crude extracts from selected plant species namely *Euphorbia candelabrum* (leaves+latex), *Euphorbia tirucalli* (leaves+latex), *Lantana camara* leaves, *Tephrosia vogelii* leaves, *Capscum anuum* fruits, *Capscum chinense* fruits, *Jatopha curcas* seeds and *Ricinus communis* seeds to identify the suitable one. The crude extracts were evaluated under laboratory conditions by dressing to maize seeds at concentration 150, 200, 250 and 300 g or ml kg^{-1} each. Each concentration was individually subjected to a total of 10 rats (5 females+ 5 males) under ''choice'' and ''no-choice'' cage experiments and observed for consecutive 7 days. Results revealed that *Ricinus communis* was effective against *Mastomys natalensis* for all seven days of the study by reducing rodent damage to 93% followed by *Japtopha curcas* and *Capscum chinense*. It was therefore, suggest that *Ricinus communis* be used by small scale maize farmers for controlling *Mastomys natalensis* at sowing stage at a concentration of 150 g dressed in one kilogram of maize seeds. However, a study on rodent behavior responses is suggested for verification of the effectiveness for comparison of *Ricinus communis* with the less effective pesticidal plants. Also a study on the effect of *Ricinus communis* on maize seed germination is suggested to be carried out.

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Do avian and terrestrial predators empty self-service traps for common vole (*Microtus arvalis*)?

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The common vole's (*Microtus arvalis*) population cycle is defined by an extreme increase in population size within two to five years that results in an outbreak with up to 1,000 common voles per hectare, followed by a population breakdown. During outbreaks voles migrate from their primary habitat, undisturbed grasslands, to secondary farmland habitat, e.g. cereals or rapeseed. Farmers often use rodenticides to manage high common vole densities. Population dynamics studies indicate that the effectiveness of rodenticide application can suffer from asynchronous management action, re-colonisation and other issues. Non-chemical methods can be efficient on small-scale but organic farmers need a large-scale applicable approach. Involving the best vole predators could offer an additional tool for rodent management that is suitable for organic farming. We tested two types of traps catching voles and emptied by foxes, racoons, cats, raptors and other birds. We developed one trap with a triangular shape to fit in a ploughed furrow at the refuge-crop interface. The other trap (standby-box, Andermatt Biocontrol AG) has a lid that can be opened by terrestrial predators to remove captured rodents. In field studies, we tested with camera traps how frequently the two trap types were emptied by predators. Our newly developed trap was emptied more often and by a more diverse group of predators than the standby trap. Only house cats (*Felis silvestris catus*), racoons (*Procyon lotor*) and foxes (*Vulpes vulpes*) were recorded opening the lid of the standby-box to remove rodents. From the new trap type, voles were additionally removed by stoats (*Mustela erminea*) and a variety of raptors and other birds. Additionally, its opening allows several rodents to jump out of the trap. If we can answer the question, if predators learn to patrol field margins, our new trap can be an effective and useful tool of an ecologically-based management.

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Research progresses on the anti-fertility effects of a contraceptive bait of quinestrol and levonogestrel (EP-1)

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Rodent pest damage is still a severe problem in the world. Due to increasing rodenticides resistance, high risk to non-target animals or people, rapid population recovery and public concerns of traditional killing, fertility control as a non-lethal and sustainable approach has been proposed as an alternative of rodent management. However, fertility control often suffers disadvantages of low palatability, repeated baiting or environmental safety problems, we are still lacking of efficient approaches of fertility control. In this presentation, we will present a review about the research progresses of the anti-fertility effects of a rodent contraceptive bait with quinestrol and levonogestrel (EP-1), mostly in China. During past decades, baits containing quinestrol and levonogestrel have been shown to have effective anti-fertility effects on various wild rodent species in both laboratory and field conditions. In laboratory experiments, EP-1 showed significant anti-fertility effects in both male and females of rodents, including greater Mongolia gerbils, Brandt's voles, midday gerbils, etc. In field tests, a single baiting of EP-1 baits ranging from 10 to 50 ppm during the breeding season could significantly reduce the reproduction and population density for several months in several rodent species, including plateau pikas, Djungarian hamster, greater Mongolia gerbils, etc. Further studies indicated that the half–lives of quinestrol and levonogestrel in water and soil were short, ranging from a few hours to about 2 weeks, suggesting that these compounds were easily degraded by bacteria in natural condition. Studies also indicated that EP-1 had minor negative effects on populations and diversity in birds. In conclusion, we believe that EP-1 is very promising for practical use for rodent fertility control. More efforts are needed to test its effects on rodent species in other countries outside China.