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CRISPR-Based Gene Drives for Pest Control

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1 **CRISPR-Based Gene Drives for Pest Control**

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9 ***Keywords:*** Gene drive, CRISPR, genetic engineering, pest control

Abstract

CRISPR-based gene drives could be employed to spread desirable genetic elements through wild populations. With the imminent development of this technology in vertebrates, we believe it is timely to highlight two forms of sex-ratio distorting gene drives that show potential as pest management tools.

In agriculture and wildlife, invasive pests are well known: rabbits and cane toads in Australia, mink in the United Kingdom, and the omnipresent infestation of rodents around the globe. In the United States alone it is estimated that introduced rats cost the economy more than $27 billion per year [1]. To counter the impact of vertebrate pests, control measures are deployed which include shooting, poison baiting, trapping and the release of biological agents (Figure 1A). These methods are costly and inadequate, and they often lead to unwanted suffering in both target and non-target species [2]. Gene drives (GDs) may offer a more cost-effective, humane and species-specific alternative than current approaches.

Gene drives for sex-ratio distortion

The gene drive concept has emerged from observations that naturally occurring selfish genetic elements, such as homing-endonuclease genes and transposons, are preferentially inherited at frequencies greater than predicted by Mendelian inheritance. This 'super-Mendelian' inheritance allows these elements to drive through a population even if they reduce the fitness of an individual organism [3] (Figure 1B). The recent discovery and repurposing of RNA-guided CRISPR endonucleases into a set of gene editing tools (Box 1)

33 allows the development of synthetic GDs in a standard molecular biology laboratory [4].

34 One potential application of GD is to distort the sex-ratio of a population. By skewing the
35 sex-ratio away from the favored Fisherian ratio of 1:1 male to female it is possible to
36 manipulate a population's reproductive performance. In most pest species, female
37 procreative capacity is responsible for maintaining the overall population size. Therefore, an
38 efficient means of population suppression is to bias the sex-ratio in favor of males [5]. A
39 grossly male population will result in a population decline, whilst an all-male population will
40 lead to eradication. Here we describe two forms of CRISPR-based sex-ratio distorting GDs -
41 homing GD and X-shredder (XS) – both of which have the potential to drive maleness. To
42 date, these GD systems have only been engineered in proof-of-concept studies in
43 mosquitoes [6, 7], with the focus on controlling vector-borne diseases. After insects,
44 invasive vertebrate pests are likely to be the next GD target.

45 **Homing gene drive targeting female fertility**

46 A homing GD works by copying or 'homing' itself into a target site in the genome. To build a
47 CRISPR-based homing GD, an animal is engineered with a GD cassette that expresses a
48 CRISPR endonuclease, such as Cas9, and one or more gRNAs (Box 1) from one allele that can
49 cut at a conserved target site on the sister allele on the homologous chromosome (Figure
50 1C). After CRISPR-mediated cleavage, homology directed repair (HDR) results in the CRISPR
51 machinery and any additional payload included in the GD cassette being copied onto the
52 homologous chromosome [4, 8]. This process ensures homozygosity for the GD cassette.

53 Targeting a homing GD to a haplosufficient female-fertility gene (HFFG) can be used to
54 disrupt the gene's coding sequence, rendering homozygous female offspring infertile; whilst

55 males and heterozygous females will retain normal fertility. Importantly, for this strategy to
56 drive through a population the homing event should occur only in germline cells that are
57 precursors to sperm or eggs. This can be achieved by using a germline-specific promoter to
58 express Cas9. By restricting homing to the germline, this will initially allow rapid spread of
59 the GD and an accumulation of fertile heterozygous GD animals that produce mostly GD
60 gametes [7, 9] (Figure 1C). As mating between heterozygous GD carriers becomes
61 increasingly likely, the population will decline due to infertility of the homozygous GD
62 female offspring, which are homozygous null for the HFFG [9]. With every generation, the
63 sex-ratio will become more biased towards males, eventually resulting in a population crash.
64 Hammond and colleagues [7] developed this system in mosquitoes and achieved
65 transmission rates of 91.4 to 99.6% in caged populations. In theory, homing GDs could be
66 adapted to control most vertebrate pests and several groups are currently undertaking pilot
67 studies in mouse models.

68 **X-shredder**

69 In XY heterogametic species, an XS is a type of sex-ratio distorting GD that cuts the X-
70 chromosome at multiple sites during spermatogenesis, thus shredding the X-chromosome
71 beyond repair [8]. To engineer a CRISPR-based XS, an XS cassette is inserted within a neutral
72 intergenic region of the Y-chromosome. The cassette encodes Cas9, which is expressed
73 under the control of a spermatogenesis-specific promoter, and one or more gRNAs that
74 target conserved repetitive sequences unique to the X-chromosome. As most X-
75 chromosomes are destroyed during spermatogenesis, the majority of sperm that mature
76 and reach the oocyte are Y-bearing, resulting in a biased sex-ratio in favour of males. By

77 placing the XS cassette on the Y-chromosome, all male offspring will inherit the cassette and
78 continue transmitting the XS to subsequent generations.

79 A CRISPR-based XS has been engineered in mosquitoes, although the system was
80 commendably safeguarded by expressing the XS cassette from an autosome instead of the
81 Y-chromosome. With this approach, Galizi and colleagues [6] achieved male bias among
82 progeny ranging from 86.1% to 94.8% in laboratory contained mosquito populations.
83 Although successful in mosquito, technical challenges facing the adaption of an XS into
84 vertebrates include identifying appropriate spermatogenesis-specific promoters in target
85 species and the transcriptional silencing of mammalian sex chromosomes during meiosis.
86 The latter may hinder expression of Cas9 from the Y-chromosome, as well as the
87 endonuclease's accessibility to shred the X-chromosome.

88 **Drive resistance and inactivation strategies**

89 The two forms of GDs described above are self-perpetuating and, in theory, would only
90 require the release of a small number of engineered animals to initiate drive. The duration
91 and extent of spread would be limited by naturally arising resistant alleles that prevent
92 CRISPR-mediated cleavage. Resistant alleles could exist in the population prior to release or
93 originate from indels generated when CRISPR-mediated cleavage is repaired by the error-
94 prone NHEJ pathway and alters the gRNA recognition sequence [8]. The rate of NHEJ-
95 mediated repair will be dependent on the species, target site and the stage of development
96 that DNA cleavage occurs. As natural selection tends to favour equal sex ratios, resistant
97 alleles that restore function would spread rapidly through the population [10].

98 Of the two strategies presented here, the XS should be less prone to inactivation by

99 resistant alleles as it targets multiple sites and, therefore, would require an animal to
100 simultaneously acquire multiple resistant alleles to incapacitate the drive. Following a
101 similar approach, it has been suggested that the evolutionary stability of homing GDs may
102 be improved by using multiple gRNAs closely spaced along the target region [4]. To test this
103 hypothesis, Prowse and colleagues [9] used *in silico* modelling to demonstrate that multiple
104 gRNAs are necessary for homing GDs to evade drive resistance and successfully suppress
105 vertebrate pest populations. Even if drive resistance were to prevail, it would be possible to
106 release a second GD targeting a different gene to continue suppressing the population.

107 Conversely, if a GD was not limited by naturally arising resistance, it would have the
108 potential to spread indefinitely through a species. Therefore, it is essential to have
109 strategies in place that could deliberately inactivate a GD that escaped containment or was
110 causing unforeseen impacts. Fortunately, both homing GD and XS systems can be
111 inactivated by the release of animals bearing engineered functionally resistant alleles or a
112 reversal gene drive which immunizes the animal against the original drive [4, 8]. However, it
113 is important to recognise that with the current technology, once either of these systems are
114 released, complete reversion to a wild-type genotype would not be possible as residual Cas9
115 and gRNA would still be present.

116 **Risks and benefits**

117 Genetically engineered animals normally come with few ecological risks. Most engineered
118 traits are for human benefit and will not be favoured by natural selection. In contrast, GDs
119 can spread through populations even if they reduce the fitness of each carrier animal [4].
120 This gives GDs more scope to escape the target population and unintentionally effect
121 extraneous ecosystems. However, the potential benefits of GDs are equally as impactful as

122 the risks. GDs could revolutionise public health, agriculture, and as discussed here, be
123 applied for pest control and ecological restoration. In line with the recent decision at the
124 United Nations Convention on Biodiversity, we believe that the potential benefits of GDs
125 warrant further investigation.

126 **Future outlook**

127 For the first time, we have the makings of a technology that could reduce or eliminate a
128 pest population in a humane and species-specific manner. If proven effective, the decision
129 to deploy a GD should be based on substantiated research and involve public engagement
130 to ensure there is societal consensus. With the rapid progress in this space, the risks
131 associated with current GD architectures are likely to be reduced with the realisation of self-
132 limiting GD strategies [11].

133

134

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137 Commission.

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144 ***Box 1. CRISPR-Cas9***

145 The CRISPR-Cas prokaryotic immune defence system, clustered regularly interspaced short
146 palindromic repeats (CRISPR) and its associated proteins (Cas), has been repurposed into a
147 set of gene editing tools. Currently, the CRISPR-Cas9 system from *Streptococcus pyogenes* is
148 the most widely used CRISPR system for genetic manipulation. The system consists of two
149 components: a guide RNA (gRNA) and a non-specific endonuclease (Cas9; Figure I). The
150 gRNA includes an 89 nucleotide 'scaffold' sequence which Cas9 binds to and a 20 nucleotide
151 user-defined 'targeting' sequence that delivers the endonuclease to the correct site in the
152 genome by Watson-Crick base pairing with the target sequence. Provided that the genomic
153 target is immediately adjacent to a protospacer adjacent motif (PAM; 5'-NGG-3' for *S.*
154 *pyogenes* Cas9), the endonuclease cuts the DNA generating a double-stranded DNA break
155 (DSB). The cells' natural DNA repair machinery then recognises the DNA is damaged and
156 repairs the DSB by one of two pathways: (1) in the presence of a DNA repair template, such
157 as the homologous chromosome or an exogenous DNA template, the DSB is repaired
158 through high-fidelity homology directed repair (HDR). HDR can be used to make accurate
159 repairs or precisely edit the DNA sequence. (2) In the absence of a DNA repair template, a
160 DSB is repaired by the error-prone non-homologous end joining (NHEJ) pathway. Repair by
161 NHEJ yields deletion or insertion mutations (indels) [12].

162

163 **Figure I. CRISPR-Cas9 gene editing.** The Cas9 endonuclease is guided to the target sequence
164 in the genome by the guide RNA (gRNA). At the target site, Cas9 cleaves the DNA creating a
165 double-stranded DNA break (DSBs). A DSB induced by Cas9 can be repaired by homology
166 directed repair (HDR) or non-homologous end joining (NHEJ). HDR can precisely repair or

167 edit the DNA sequence. NHEJ-mediated repair produces insertion or deletion mutations
168 (indels).

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171
172 **Figure 1. CRISPR-based gene drive strategies for controlling vertebrate pests.** (A) Invasive
173 pests are a global concern. Current control strategies are inhumane, costly and often
174 inadequate. (B) Left: Mendelian inheritance of an altered gene. Right: inheritance of a gene
175 drive. (C) A homing gene drive (GD) targeting female fertility. In the first generation,
176 heterozygous (Het) GD animals are released carrying a GD cassette (purple) which disrupts
177 the coding sequence of a haplosufficient female-fertility gene (HFFG; yellow). Within the
178 germ cells, the GD cassette expresses Cas9 and gRNA(s) that cut the HFFG on the wild-type
179 (WT) chromosome. The germ cells then repair the cut by homology direct repair (HDR),
180 using the GD chromosome as the repair template. This process copy's the GD cassette onto
181 the WT chromosome and ensures that most sperm or eggs carry the GD cassette. Matings
182 between Het GD and WT animals will give rise to an increasing number Het GD animals. In
183 subsequent generations, as mating between Het GD animals becomes increasingly likely,
184 the population will decline through infertility of homozygous (Hom) GD female offspring,
185 which are homozygous null for the HFFG. (D) X-Shredder (XS). During spermatogenesis, Cas9
186 and gRNA(s) are expressed from the XS cassette (orange) located on the Y-chromosome (Y)
187 and shred the X-chromosome (X) beyond repair. The majority of sperm that mature and
188 reach the oocyte are Y-bearing, resulting in most offspring being XS males.

189 ***References***

190 1 Pimentel, D. (2007) Environmental and Economic Costs of Vertebrate Species Invasions
191 into the United States. In *Managing Vertebrate Invasive Species: Proceedings of an*
192 *International Symposium* (Witmer, G.W.*, et al.*, eds)

193 2 National Academies of Sciences, Engineering, and Medicine (2016) *Gene Drives on the*
194 *Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public*
195 *Values*. The National Academies Press

196 3 Burt, A. and Trivers, R. (2009) *Genes in conflict: the biology of selfish genetic elements*.
197 Harvard University Press

198 4 Esvelt, K.M.*, et al.* (2014) Concerning RNA-guided gene drives for the alteration of wild
199 populations. *eLife* 3, e03401

200 5 Hamilton, W.D. (1967) Extraordinary sex ratios. A sex-ratio theory for sex linkage and
201 inbreeding has new implications in cytogenetics and entomology. *Science* 156, 477-488

202 6 Galizi, R.*, et al.* (2016) A CRISPR-Cas9 sex-ratio distortion system for genetic control.
203 *Science Reports* 6, 31139

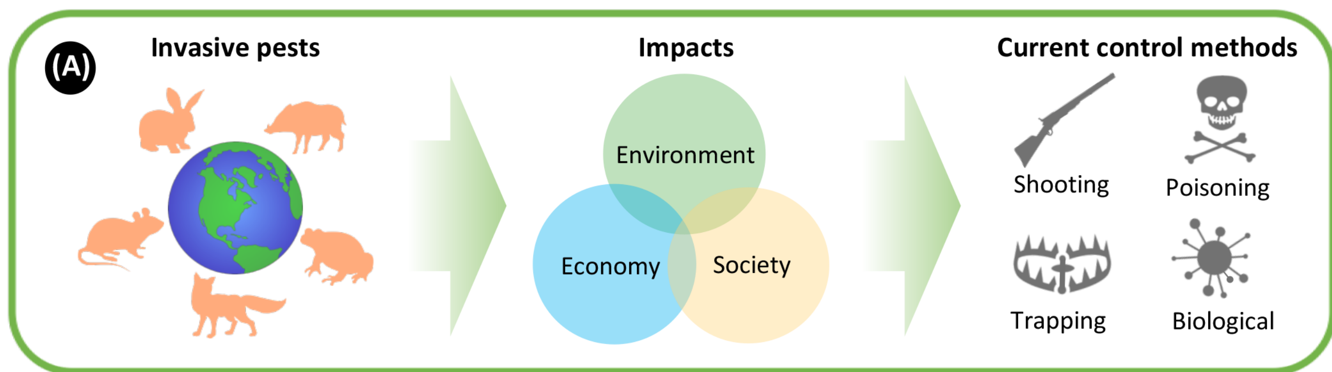
204 7 Hammond, A.*, et al.* (2016) A CRISPR-Cas9 gene drive system targeting female
205 reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nature biotechnology* 34,
206 78-83

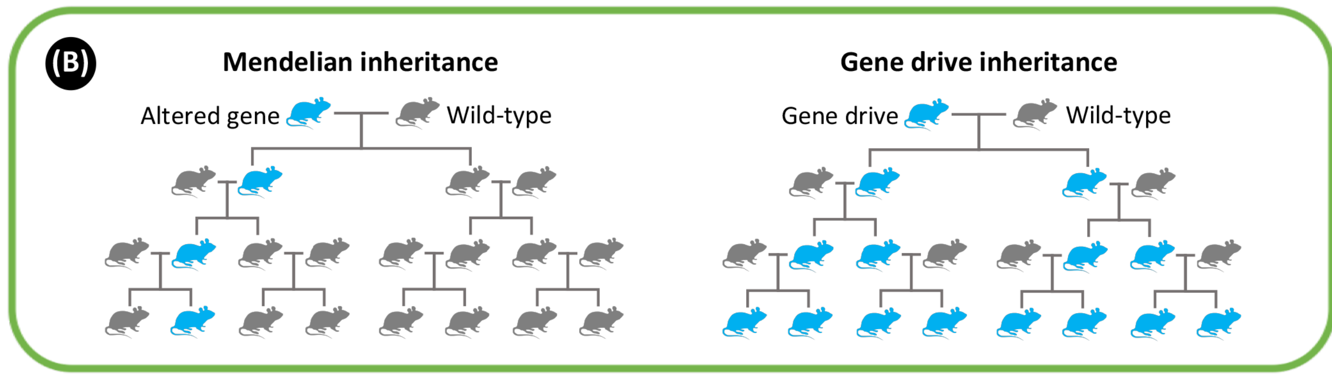
207 8 Champer, J.*, et al.* (2016) Cheating evolution: engineering gene drives to manipulate the
208 fate of wild populations. *Nature Reviews Genetics* 17, 146-159

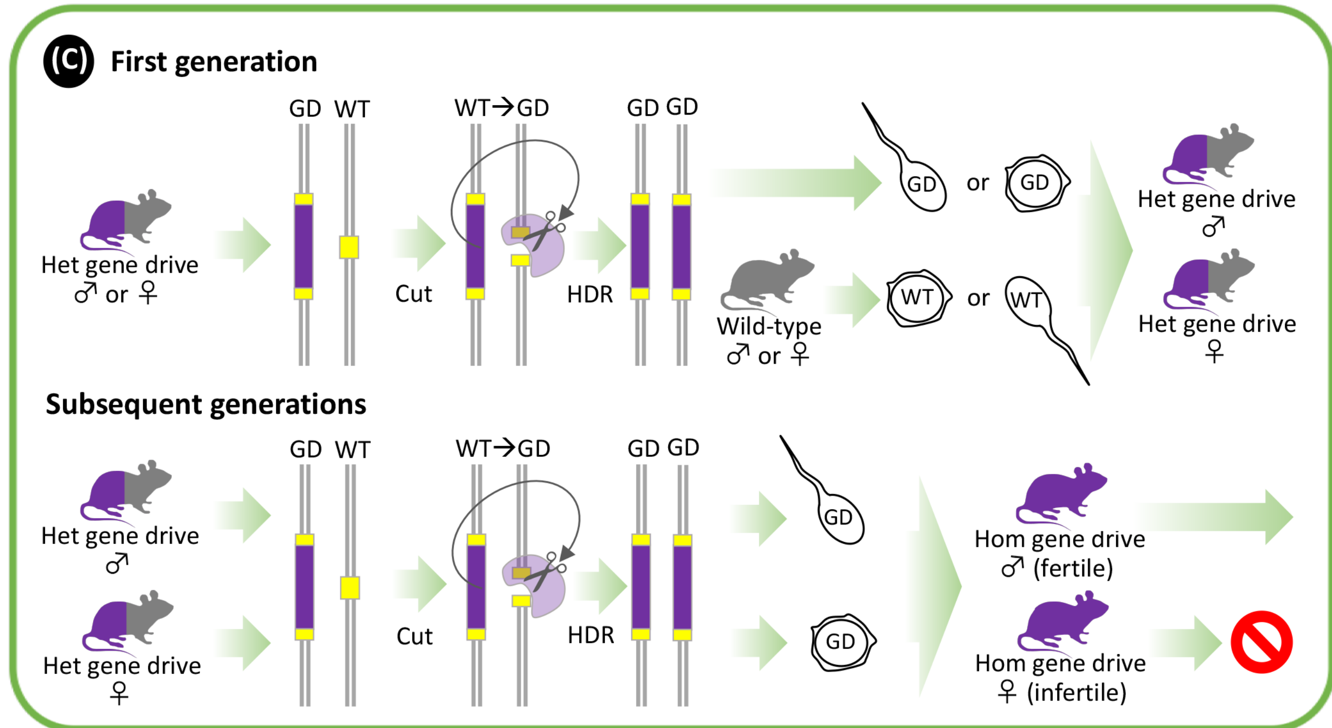
209 9 Prowse, T.A.A.*, et al.* (2017) Dodging silver bullets: good CRISPR gene-drive design is
210 critical for eradicating exotic vertebrates. *Proceedings of the Royal Society B: Biological*
211 *Sciences* 284

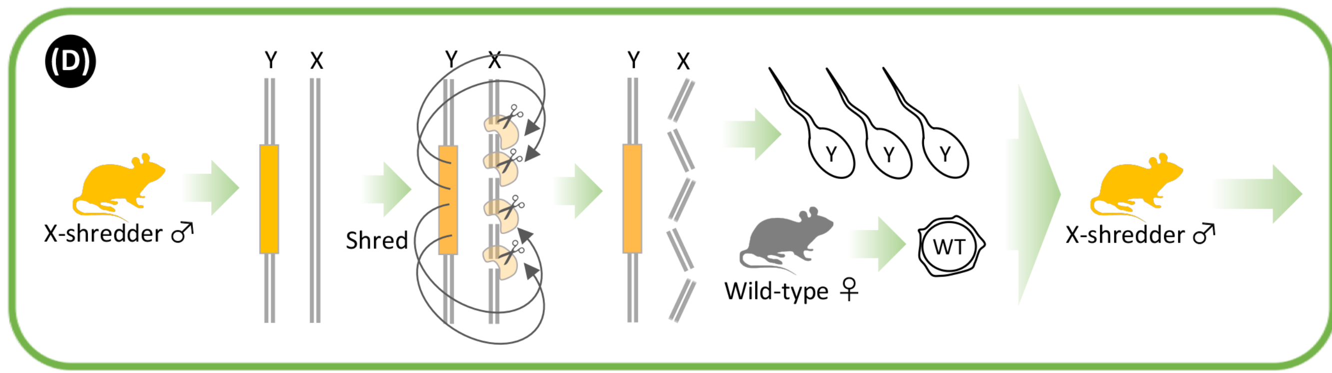
212 10 Hammond, A.M., *et al.* (2017) The creation and selection of mutations resistant to a gene
213 drive over multiple generations in the malaria mosquito. *bioRxiv* DOI: 10.1101/149005
214 11 Noble, C., *et al.* (2016) Daisy-chain gene drives for the alteration of local populations.
215 *bioRxiv* DOI: 10.1101/057307
216 12 Cong, L., *et al.* (2013) Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science*
217 339, 819-823
218

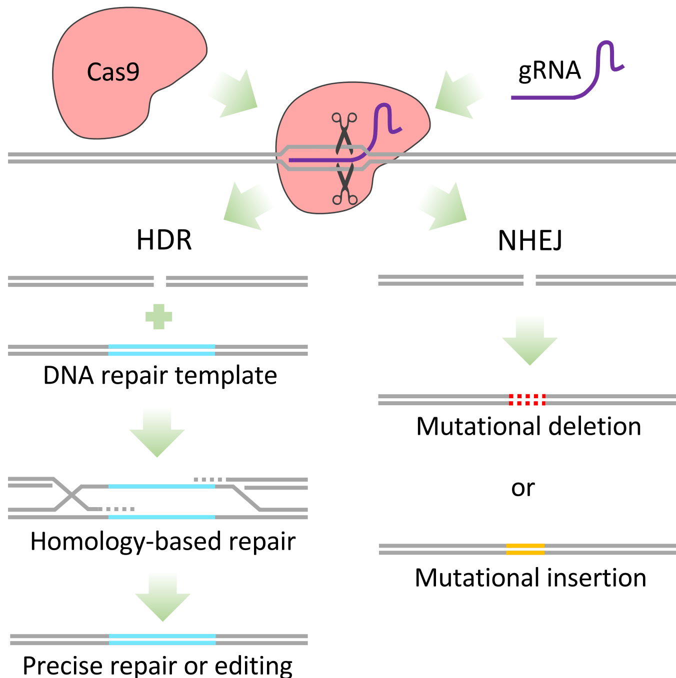
Revised Figure 1 - PDF










Cas9
gRNA
HDR
NHEJ
DNA repair template
Homology-based repair
Precise repair or editing
Mutational deletion
or
Mutational insertion