Trialling gene drives to control invasive species: what, where and how?

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Abstract The control of invasive species would be enhanced through the addition of novel, more effective and sustainable pest management methods. One control option yet to be trialled in the field is to deploy transgene-based 'Gene Drives': technologies which force the inheritance of a genetic construct through the gene pool of a wild population, suppressing it or replacing it with a less harmful form. There is considerable interest in applying gene drives to currently intractable invasives across a broad taxonomic range. However, not all species will make efficient or safe targets for these technologies. Additionally, the safety and efficacy of these systems will vary according to where they are deployed, the specific molecular design chosen, and how these factors interact with the ecology of the target pest. Given the transformative but also controversial nature of gene drives, it is imperative that their first field trials are able to successfully demonstrate that they can be used safely and efficiently. Here, we discuss how to maximise the probability of this outcome through considering three important questions: *What* types of invasive species should we use to trial gene drives? *Where* should we be trialling them? and *How* should these trials be conducted? In particular, we focus on the ecological, genetic and geographic features of small, isolated islands which make them ideal locations for these initial trials. A case study of an island invasive that is deemed highly appropriate for gene drive intervention, and for which gene drive development is currently underway (*Mus musculus*), is used to further explore these concepts.

Keywords: biodiversity conservation, CRISPR, *Culex quinquefasciatus*, gene drive, island invasive, *Mus musculus*, population eradication, restoration

INTRODUCTION

Molecular advancements have made feasible a new range of Genetic Pest Management (GPM) strategies – the transgene-based gene drives (Sinkins & Gould, 2006). These technologies aim to introduce DNA sequences (the gene drive transgene) into the genome of a wild pest population through the release of genetically engineered individuals which go on to mate with conspecifics in the field. Once introduced, the inheritance of the gene drive is forced – driven – through the target population gene pool along with its control phenotype. This driving effect can be achieved, for example, by biasing inheritance of the transgene above normal mendelian levels, or through placing an evolutionary advantage on inheritance of the transgene at the population level. Proposed control phenotypes aim either to reduce/eradicate a pest population – "population suppression" strategies – or to leave a population intact but modify it so that it is less harmful (e.g. by spreading a transgene which makes a mosquito population less able to transmit a particular disease) – "population replacement" strategies (Alphey, 2014). Within population suppression, current proposals aim to spread either a sex ratio bias (usually in favour of males) or a genetic load, e.g. female sterility (Deredec, et al., 2008).

Theoretically, gene drives could be engineered that are capable of spreading to every member of an interbreeding population from one or several relatively small initial releases (Deredec, et al., 2008). This autonomous nature is appealing for invasive species control, where programmes often extend into remote/inaccessible areas and less than total eradication may be viewed as failure. Indeed, there is increasing interest in applying gene drives to currently intractable invasive species that threaten biodiversity (Alphey, 2002; Gould, 2008; Esvelt, et al., 2014; Simberloff, 2014; Thresher, et al., 2014; Campbell, et al., 2015; NASEM, 2016; Harvey-Samuel, et al., 2017; Piaggio, et al., 2017). However, two primary concerns arise from their proposed use. Firstly, that a gene drive transgene could unintentionally spread beyond a target geographic area (e.g. from an invasive population into the native range of the invader) or into a non-target species through hybridisation/horizontal-gene transfer – here collectively termed 'transgene escape'. Secondly, that their persistence, once released, could cause unintended ecological effects that are difficult to reverse (Sutherland, et al., 2014; Webber, et al., 2015; NASEM, 2016).

Previous field testing of gene drives is limited to non-transgenic population replacement utilising artificial infections of *Aedes aegypti* mosquitoes with the intracellular bacterium *Wolbachia* (Hoffmann, et al., 2011; Schmidt, et al., 2017). *Wolbachia* technologies are considered non-transgenic as they do not, deliberately, involve the introduction of DNA sequences into the target pest genome. Proposed application of transgene-based gene drives to invasive species differs from *Wolbachia* in that the systems available are, potentially, significantly more powerful and flexible and their taxonomic scope is broader, encompassing groups as divergent as plants, mammals, fish and molluscs, in addition to insects (Gould, 2008; Hodgins, et al., 2009; Thresher, et al., 2014; Campbell, et al., 2015; Sytsma, et al., 2015; Webber, et al., 2015). The first open-field trials of transgene-based gene drive technologies will thus represent a precedent-setting milestone. As recommended by the USA National Academy of Sciences (NASEM, 2016) , these trials will seek to examine whether the efficacy (e.g. its ability to invade a target population and induce a desired control phenotype therein) and safety (e.g. our ability to constrain its spread to the target population using molecular or experimental designs) of a gene drive system conform with theoretical expectations, themselves informed by preliminary laboratory experiments and mathematical modelling (Benedict, et al., 2008; Brown, et al., 2014). As such, open-field trials can be considered extensions of initial highly biocontained laboratory experiments where artificial biocontainment (Akbari, et al., 2015) is 'relaxed' because aspects of efficacy and safety have previously been demonstrated. Both these aspects – efficacy and safety – are important in order to convince a potentially sceptical public that they may have confidence in the wider use of these technologies.

Here we summarise the primary considerations involved in conducting the precedent-setting open-field trials of transgene-based gene drives (henceforth 'gene

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drives') in invasive species through posing three questions (1) What types of invasives are appropriate targets for these trials? (2) Where should the trials of these systems be located? (3) How should these trials be conducted? These questions are considered with the aim of exploring how these technologies could be trialled against invasive species as efficaciously as possible, whilst minimising the risk of transgene escape. In order to increase the value of this discussion these points are addressed in a general, rather than taxon-specific manner. Additionally, we explore their implications for a specific invader currently being targeted for control using gene drives – the house mouse, *Mus musculus* (See case study: GBIRd and Table 1). We bring this forward with the purpose of encouraging dialogue and improving criteria for such trials.

WHAT CHARACTERISTICS ARE IMPORTANT WHEN CHOOSING A TARGET ORGANISM?

General characteristics of a gene drive target

Minimum *requirements* for gene drive development are that the target pest is sexually reproductive, is amenable to laboratory rearing/germ-line transgenesis and is genetically well characterised.

As barriers to gene-flow within a population will decrease the efficiency of a gene drive's spread (see: The importance of dispersal), target species should preferably be obligately sexually reproductive (Alphey, et al., 2010) and incapable of self-fertilisation, which may simultaneously reduce the potential for gene drive resistance evolution (Bull, 2016). As such, it is unlikely these systems will be broadly applicable to invasive plants, of which many propagate vegetatively or through self-fertilisation (Kolar & Lodge, 2001; Rambuda & Johnson, 2004). Regarding transgenesis, the ease with which the germ-line cells can be manipulated will influence the speed that new transgene designs can be tested. Insect transgenesis has predominantly been through microinjection of pre-blastoderm embryos which requires that the fertilised egg is accessible. Transformation of species which are viviparous (e.g. the tsetse fly) or whose embryos are laid in inaccessible protective structures (e.g. pods or cases) may prove more challenging (Bourtzis, et al., 2016). Finally, as gene drives require the expression of various genetic components in highly temporal or spatially explicit patterns, often to target precise genomic loci, a good knowledge of the genetics of a target, e.g. a high-quality genome/transcriptome sequence and an understanding of the molecular-genetic basis of sex determination, is imperative.

Desirable characteristics are not absolutely necessary for gene drive development but, in practice, species whose biology diverged significantly from these characteristics would be deemed as inappropriate targets for these technologies.

Chief amongst desirable characteristics is a short generation time. This will minimise the time taken for strain development, and for these vertically transmitted systems to spread through and control a target population. Similarly, species with complex mating systems (e.g. the synchronised and ephemeral mating events of termites or ants) or where subsets of the population can remain dormant and inaccessible (e.g. long-term seed banks) effectively

Table 1 Idealised ecological selection criteria proposed as an initial filter for potential trial islands for potential gene drive constructed mice trials within Australia, New Zealand, USA. Additional steps will be required prior to any potential field trial, including engagement with stakeholders (e.g. land managers, local communities) and regulators to determine final approval.

Criteria	Rationale
1 Island is biosecure Desktop assessment indicates: a. Closed to public or infrequent/controlled visitation b. Remote enough (>1 km from other land masses) to avoid unassisted immigration or emigration	● Mice typically invade remote islands through human mediated transport, not through swimming (Russell & Clout, 2005). ● *M. musculus* are known to have swum up to 500 m between land masses (Harris, et al., 2012). ● Closed population required for proof-of-concept ● After desktop assessment. If the island passes other filters and is tentatively selected, conduct a biosecurity risk assessment. Island biosecurity plans for individual islands or island groups should be developed and implemented if island is selected (Fritts, 2007; Russell, et al., 2008; AAS, 2017)
2. No significant challenges exist to treatment using traditional methods to eradicate mice, e.g.: a. Uninhabited (besides research station or similar) b. No livestock c. No native rodents d. No non-target species of concern e. Regulatory environment allows the use of brodifacoum bait products and no rodenticide resistance alleles present f. Island size <300 ha g. Single land manager	● Provides a means to terminate experiments (i.e. exit strategy) using traditional methods without known complicating factors.
3. *M. musculus* are the only rodent present *or* could be introduced.	● Mouse behaviour is known to change significantly in the presence of rats (Harper & Cabrera, 2010). ● There may be man-made or other islands that are suitable that don't currently have *M. musculus* present.
4. Reasonably economical and feasible to visit the island year-round.	● Some islands are cost prohibitive to visit. ● Seasonal conditions may impact safe access to the island.

extend the generation time and may limit transgene introgression into or through a wild population (Alphey, et al., 2010). Furthermore, it is critical that there is a good knowledge of the ecology (e.g. mating systems, population dynamics and community interactions) of the target and in the case of vectors, the ecology and epidemiology of the pathogen and disease. The importance of this knowledge when developing a GPM strategy – from choosing the most appropriate/effective system, to predicting the impact of a strategy on a target population and community – cannot be overstated (Yakob, et al., 2008; Bax & Thresher, 2009; Yakob & Bonsall, 2009; Bonsall, et al., 2010; Thresher, et al., 2013; Piaggio, et al., 2017). Finally, it is desirable that the target is the dominant and ideally, sole, cause of an impact. As these strategies are vertically transmitted, they are extremely species-specific, making scenarios where there are multiple contributors to an impact (e.g. the spread of avian pox in Hawaii, where there are both mechanical and vector-based disease transmission routes) less appropriate.

The importance of dispersal

Gene-flow between populations

In limiting transgene escape into non-target areas, two important and interacting considerations are the level of gene-flow between a target and non-target population and the invasion threshold of the gene drive deployed (Figs 1 and 2) (Marshall & Hay, 2012). The invasion threshold is the theoretical frequency a gene drive transgene must be present at in a population before it will begin to spread. Highly invasive gene drives spread from very low invasion thresholds (e.g. the introduction of a few individuals into a target population) while less invasive systems may require significant levels of introduction before they begin to spread (high invasion threshold). Transgene escape may be considered an issue if gene-flow occurs at a frequency which makes it probable that a gene drive will exceed its invasion threshold in a non-target population

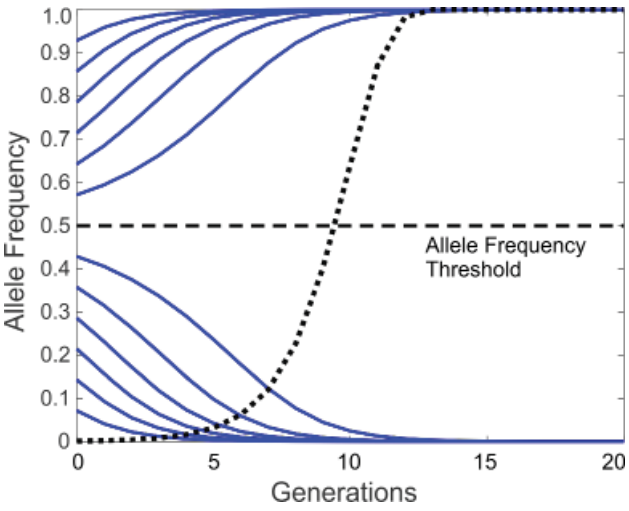

Fig. 1 Gene drives may be classified by their level of invasiveness, which is defined as the frequency they must reach in a target population before they begin to spread (the invasion threshold).Relatively non-invasive gene drives such as underdominance-based systems (Reeves, et al., 2014) (solid lines) require a high minimum allele frequency (dashed line) to be exceeded before they will begin to spread (50% of the population in this simulation). This differs from highly invasive (also known as "global") gene drives such as homing-based systems (Deredec, et al., 2008; Unckless, et al., 2015) (dotted) that will theoretically spread throughout a population even from a very low initial allele frequency, at least in the absence of resistant alleles.

within the time-frame of a trial (Akbari, et al., 2013). An 'acceptable' level of gene-flow between target and non-target populations will therefore be significantly higher for less invasive gene drives (Fig. 2). As the choice of gene drive may be constrained by the desired outcome (less invasive systems are generally more suited to replacement rather than suppression), it may not always be possible to choose less invasive designs to prevent transgene escape in species which are capable of long-distance gene-flow. A more flexible option is to trial gene drives in species which show limited ability to disperse and where human-mediated dispersal pathways can be managed. As previously noted (NASEM, 2016), important considerations here are the distance, frequency and life-stage of dispersal. Generally, species which disperse as juveniles/adults will show lower rates of gene-flow between populations than those which disperse as fertilised embryos (seeds or spores) or gametes (e.g. wind-borne pollen) (NASEM, 2016). Furthermore, dispersal via gametes may be more likely to result in interspecific hybrids, potentially increasing the risk of transgene escape into non-target species (NASEM, 2016). Consideration of these dispersal issues may make terrestrial animals more attractive targets than plants or marine species. As social interactions can strongly influence adult/juvenile dispersal events, it is important to consider how the predicted outcome of a particular gene drive may interact with these species-specific behavioural cues. For example, mate-limitation or increased inbreeding at low population densities or highly skewed sex ratios (both expected outcomes of proposed suppression gene drive designs) could in some species/scenarios result in increased levels of dispersal (Clobert, et al., 2012; Matthysen, 2012) and potentially also transgene escape.

Prior knowledge of the dispersal behaviour of an invasive population is therefore a prerequisite to safely deploying a gene drive. Fortunately, for many important invaders details of their dispersal mechanisms, invasion rates and levels of gene-flow within their invaded range already exist – in addition to other useful details such as the observed variance in their population size. Potential target species and populations could be short-listed based on the existence of this historical information, which could then be used to inform models predicting the potential for transgene escape during the expected time-frame of a trial.

Gene-flow within a population

Reaction-diffusion models have shown that dispersal rates will affect the speed that a gene drive travels through a target population (Beaghton, et al., 2016). Under more realistic scenarios, barriers to gene-flow within a population may have a more qualitative effect on whether a gene drive will spread or persist (North, et al., 2013). This concern could be reduced by avoiding targets whose populations show strong local spatial structuring, e.g. those which engage in high levels of sib-sib mating (Hamilton, 1967). However, even less extreme levels of spatial structuring resulting from limited life-time dispersal can significantly affect the ability of a gene drive to spread through and collapse a target population (Huang, et al., 2011; Eckhoff, et al., 2017). In particular, species whose population dynamics are significantly affected by seasonality may provide more fragmented landscapes for a gene drive to attempt to traverse. Models comparing gene drive dynamics in spatially explicit and homogenous mosquito populations suggest that increased structuring of a target population decreases the parameter space under which the target population is successfully eradicated (Eckhoff, et al., 2017). In these models, sub-populations became explicit annually in response to lowered population densities during the dry season. If sub-populations became explicit prior to arrival of the spreading transgene, these areas could act as a source for wild-type reinvasion into areas where the

drive had eradicated the pest the previous season. Although limited within-population dispersal can be overcome through increasing the 'patchiness' or number of transgenic releases (Huang, et al., 2011; Eckhoff, et al., 2017), this tactic partially negates the primary advantage of employing gene drives. In choosing a target it is thus critical to have evaluated whether, given their population spatial-structure and the gene drive chosen, the release effort required to efficiently eradicate or replace a population is low enough to justify intervention with this technology.

Relatedness to important pests

Development and trialling of gene drives against invasives will proceed most efficiently if target species impact multiple values (e.g. human or animal health, agriculture, conservation). If these 'dual-target' species can be identified then the financial burden of developing gene drive strategies could be shared amongst different funding agencies, efficient designs/components shared between different researchers and the benefits of, and motivation for gene drive deployment shared amongst varied stakeholders. If a target invasive did not impact multiple values, gene drive development would still benefit if they were closely related to species in which GPM technology had previously been investigated, due to the transferability of many underlying molecular designs and components (Harvey-Samuel, et al., 2017). Examples of dual-target species are the mosquito (*Culex quinquefasciatus*) – a vector for multiple human diseases (Eldridge, 2005) and an invasive vector of avian malaria in Hawaii (LaPointe, et al., 2012) – and rodents including the house mouse (*Mus musculus*) and rats (e.g. *Rattus exulans, R. norvegicus and R. rattus*) which collectively are serious economic pests of agriculture (Aplin, et al., 2003; Pimentel, et al., 2005), impact infrastructure, are hosts for human, domestic animal and wildlife disease (Banks & Hughes, 2012), and amongst the most damaging invasives of island ecosystems (Angel, et al., 2009; Harper & Bunbury, 2015). Encouragingly, germ-line transgenesis and genome sequences already exist for *C. quinquefasciatus* (Allen, et al., 2001; Arensburger, et al., 2010), *M. musculus* (Waterston, et al., 2002; Ivics, et al., 2014) and *R. norvegicus* (Gibbs, et al., 2004; Ivics, et al., 2014). Moreover, all these species are invasive in isolated, uninhabited areas where there are no closely related species: desirable characteristics for a gene drive trial location (see next section).

WHERE SHOULD TRIALS BE CONDUCTED?

In order to maximise containment and efficacy, small, isolated islands are ideal locations for the first trials of gene drives (WHO/TDR, 2014).

Advantages of island locations to trial safety

Limiting intraspecific transgene escape

Gene-flow from an invasive population to conspecifics in its native range will decrease with increasing inter-population distance, the ecological inhospitality of the intervening area and the size of the invasive 'source' population. Locating trials on small, isolated islands can therefore act as an ecological containment strategy (WHO/TDR, 2014; NASEM, 2016), reducing the risk of intraspecific transgene escape. The effectiveness of this containment will depend on the proximity of a trial island to the native range of an invader, its natural and human-

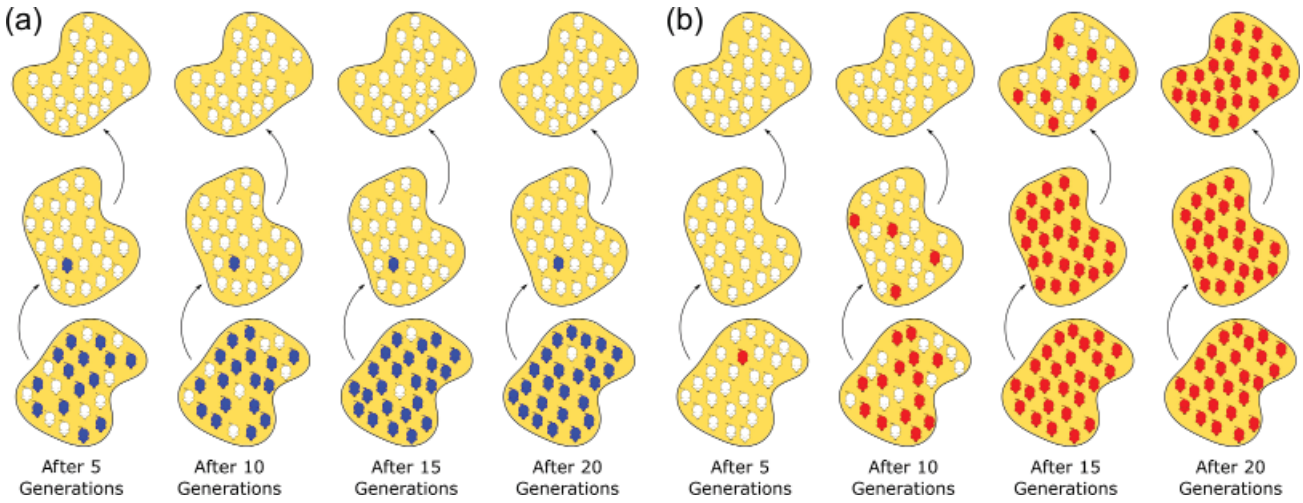

Fig. 2 The invasiveness of gene drive systems affects their containment ability once deployed in the field. Relatively non-invasive systems require large initial introductions before the gene drive will begin to spread and therefore migration alone is unlikely to exceed their invasion threshold. Highly invasive gene drives, on the other hand, can spread from only a few initial colonists and are predicted to spread through all linked populations. This is illustrated above using a three deme population genetics mathematical models. In each case we assume that a target (bottom) island and a nearby neighbour (middle) exchange 2% of their respective populations by migration in each generation while the nearby neighbour and a more remote (top) island exchange just 1%. It is assumed that no direct migration occurs between the target and remote islands due to the distance between them. Resulting transgene frequencies for each island at various times after the transgenic release are represented diagrammatically by a series of 25 mice (each representing a transgene frequency of 4%). White and shaded mice respectively represent wild-type and underdominance/homing drive transgenic allele frequencies, rounded to the nearest 4% (i.e. to the nearest whole mouse). Panel (a) shows results for a frequency dependent, single locus haploinsufficient underdominance-based system (Reeves, et al., 2014). This is a relatively non-invasive system with a high invasion threshold of 50% (See Fig. 1, solid lines). Here it is assumed that wild-type and transgene homozygotes suffer no fitness cost while 50% of heterozygous offspring are non-viable. For an initial transgene frequency of 55% it can be seen that the system spreads throughout the target population but does not reach significant levels in the neighbouring populations. Panel (b) shows results from a homing-based gene drive (Deredec, et al., 2008; Unckless, et al., 2015) which imparts no fitness cost on individuals and converts heterozygotes to homozygotes with 100% efficiency, introduced with an initial transgene frequency of 0.1%. The population genetics of this gene drive are shown in Fig. 1, (dotted line). Even this low initial frequency allows this highly invasive gene drive to spread throughout the target, and in time, the neighbouring populations also.

mediated dispersal ability and the invasiveness of the gene drive being trialled. A set of case-studies illustrating the interplay between these factors is the open-field releases of artificial *Wolbachia* infections aimed at local replacement of *A. aegypti* mosquito populations in Australia. After deliberate establishment in relatively isolated trial *A. aegypti* populations (Hoffmann, et al., 2011), it was found that long-distance dispersal was taking *Wolbachia* infected mosquitos into areas beyond the trial site (up to 1.86 km away) but that migration rates were insufficient over this distance to overcome the relatively high invasion threshold of the *Wolbachia* system (>30%) which remained largely contained to the trial site (Hoffmann, et al., 2014). Conversely, in subsequent releases where the trial site formed part of a larger, continuous *A. aegypti* population, *Wolbachia* was capable of spreading, albeit slowly, to high frequency beyond release sites and into the wild target population (Schmidt, et al., 2017). Gene drives with lower invasion thresholds than *Wolbachia* will require significantly greater isolation and/or molecular safeguard designs to limit transgenes to target populations/areas (discussed in the **How** section). This concept is illustrated for transgene-based gene drives in Figs 1 and 2.

In the context of island trial locations, the potential for a gene drive to cover large geographic distances, potentially back to mainland populations, through 'island-hopping' should not be overlooked (Bellemain & Ricklefs, 2008). For suppression drive designs, this island-hopping would require the existence of viable populations extending back to a native range and for the drive to escape each invaded 'stepping-stone' population before that population was itself eliminated by the drive. However, for replacement drives these aspects would not be a pre-requisite.

Limiting interspecific transgene escape

Transgene escape between species could take place either through horizontal gene transfer (HGT – acquisition of genetic material from an organism other than a direct ancestor) or introgression following hybridisation. Signals of HGT in metazoans can be seen by sequence comparisons between species (e.g. Crisp, et al., 2015). However, even the most frequent of these HGT events are rare, seen in nature on timescales of millions of years (e.g. Ortiz, et al., 2015). Therefore, as discussed generally for mosquitoes (Besansky, 2015) and specifically for homing-drives (Burt, 2003), HGT of a gene drive is held to be unlikely to occur at a frequency which will make it a realistic concern.

Regular gene-flow between native and invasive species through introgressive hybridisation, however, is well documented (Mooney & Cleland, 2001). Here, island locations provide both benefits and disadvantages in terms of limiting transgene escape. A benefit is that, given a frequency of fertile hybridisation events, stochastic elimination of an escaped transgene prior to its spread in a non-target species is more likely in small, island populations than at continental scales. However, hybridisation between closely related invasive and native species may be higher in insular compared to continental communities (Rhymer & Simberloff, 1996), potentially allowing transgenes to introgress into native populations at increased rates on islands. The potential genetic homogeneity of an island invasive population and simplicity of island communities (reducing the number of hybridising congeners) may prove advantageous in designing sequence-specific molecular safeguards to limit this risk.

Advantages of island locations to trial efficacy

Geographic isolation

Trials of gene drives will seek to achieve a series of pre-defined scientific endpoints (Brown, et al., 2014; NASEM, 2016). These will include evidence that the transgene is able to spread efficiently in the wild population, as well as endpoints specific to individual designs (e.g. reduced population density or reduced number of fully-competent vectors for suppression and replacement strategies, respectively). As immigration of wild-type individuals into a target population effectively dilutes the frequency of the transgene, unanticipated immigration will cause drive rates to be estimated inaccurately; this has been a frequently-observed problem in trials of sterile insects for population suppression (Klassen & Curtis, 2005) and is assumed to have prevented fixation of artificial *Wolbachia* infected mosquitoes in open-field trials (Hoffmann, et al., 2014). A sufficiently isolated island trial site will reduce this concern through minimising wild-type immigration. What constitutes 'sufficient' geographic isolation could be considered in conjunction with estimating outward gene-flow from a proposed trial island, acknowledging that migration rates between populations may not be symmetrical (Kawecki, 2004) and may only occur during infrequent events (e.g. El Niño, hurricanes).

Small population size

For equivalent release numbers/resources, introductions can be made at a higher population allele frequency on small islands than at larger, continental scales. This is primarily advantageous in testing gene drives with high invasion thresholds. However, even for more invasive systems, test releases would likely take place at frequencies well above the estimated minimum to protect against stochastic loss of the transgene in initial generations. Increased introduction rates will also allow the transgene to reach fixation (or a stable internal equilibrium) more rapidly (Deredec, et al., 2008). Moreover, for population-suppression strategies, smaller target populations may mean that density-dependent processes such as Allee effects (Tobin, et al., 2011) and environmental stochasticity (Eckhoff, et al., 2017) can be leveraged to more rapidly drive populations to extinction.

Genetically distinct

Small, insular populations arising from recent single invasion events are likely to be relatively genetically homogenous (Dlugosch & Parker, 2008). Assuming that heritable resistance to gene drives is possible (Bull, 2015), but founder individuals did not carry resistance alleles, this would provide target populations initially entirely susceptible to a released drive. Furthermore, given a constant mutation rate, such a gene drive resistance allele is less likely to arise in smaller, isolated populations within the time-frame of a trial. Conversely, however, if founder individuals did display pre-existing resistance it may occur at high frequencies. Target island populations should be screened prior to a trial for the presence of pre-existing resistance mutations; a relatively simple task for sequence-specific homing-drives, but potentially less straightforward for other technologies.

Which islands?

Islands that are small and sufficiently isolated to provide effective ecological containment could provide ideal locations for trialling gene drives. However, there are a

number of biological, geographic and social criteria which will, in general, make a location more or less suitable for trialling GPM strategies (Benedict, et al., 2008; Lavery, et al., 2008; Brown, et al., 2014) and which can be extended to identify particularly promising examples within this group.

Biological criteria

If sufficiently isolated, invasive populations will be allopatric from conspecifics in their native range but sympatric with native congenic populations with which they might hybridise. If it occurs at an appreciable frequency, interspecific gene-flow may therefore be considered the more likely of the two risks when trialling gene drives in these locations. The most effective solution would be to avoid locations where there are closely related native species. For example, targeting invasive rodents on off-shore islands in New Zealand (which has no native terrestrial mammals) would pose low/no risk of transgene escape into native species, whereas deployment of the same technology in areas with diverse endemic rodent fauna such as south-east Asian archipelagos (Amori, et al., 2008) would likely require extensive pre-trial risk assessment. A further point to consider is that hybridisation events may be unidirectional with regards to sex (Rhymer & Simberloff, 1996). Molecular designs such as Y-drive which are transmitted exclusively through the paternal line would not be introgressed into a native population if hybrids formed via crosses between native males and invasive females.

If sufficient safety measures are taken, gene drives are expected to act in an extremely species-specific manner and are thus highly suitable for deployment in ecologically sensitive locations. However, a precautionary approach would suggest that precedent-setting trials be conducted in locations devoid of endangered/threatened flora or fauna (Brown, et al., 2014). This is particularly relevant if broader spectrum conventional control methods are used to terminate the trial at a pre-defined endpoint (see Table 1).

Geographic criteria

Barriers to gene-flow will decrease the efficiency of a released gene drive. Islands with relatively simple geographies and a resulting homogenous invasive population, for example low-lying oceanic islands, will therefore be most amenable to initial trials of these technologies. Where multiple islands occur in close proximity, these areas could be used to test assumptions on the spread of a drive technology within/between populations depending on the dispersal of the target (e.g. coral atolls/archipelagos for short/longer distance dispersal, respectively).

Social criteria

Challenges associated with invasive species control in inhabited areas are well-documented (Oppel, et al., 2011; Glen, et al., 2013). The novel and controversial nature of gene drives means that these challenges are likely to be exacerbated during their first trials. Levels of regulatory/engagement costs, risk assessment and societal objection are all likely to be more favourable if initial trials take place in uninhabited areas which are not of great cultural value. At least as importantly, restricting traffic off an island during a trial will substantially reduce the likelihood of transgene escape via intraspecific gene-flow. Employing modified biosecurity measures currently employed during conventional eradication efforts (Russell, et al., 2008), this would be far more feasible for uninhabited areas.

Previous experience in choosing sites for self-limiting GPM mosquito trials suggest that two social criteria critical for site identification are the existence of a credible regulatory structure and an enthusiastic local participant (e.g. academic researcher or wildlife management agency) with expertise regarding the invasive being targeted (Brown, et al., 2014). The regulatory framework in operation is relevant at multiple stages during planning and implementing a gene drive trial, from granting importation permits for gene drive organisms (Brown, et al., 2014) to determining appropriate risk assessment (NASEM, 2016) and public engagement (Lavery, et al., 2008) activities and experimental design/biosecurity during and after a trial (Benedict, et al., 2008). A robust and defensible regulatory framework allows public confidence in approved trials and reduces the likelihood of a trial being halted prematurely due to previously unvoiced concerns (Brown, et al., 2014). As regulation of gene drive trials is expected to take place on a case-by-case basis (Oye, et al., 2014) a local participant with knowledge of the regional ecological, social, economic, political and cultural context of deployment is invaluable. Additionally, due to the relative complexity and large scale (both temporal and spatial) expected of a gene drive trial, access to experienced research teams provided by a local collaborator would likely be necessary.

How should trials be conducted?

Practical guidance on how to conduct field-trials of self-limiting GPM mosquitoes (e.g. aspects of experimental design, safety and efficiency endpoints) is available (Benedict, et al., 2008; Brown, et al., 2014) and has been extended to the case of gene drives (WHO/TDR, 2014; NASEM, 2016). We will not replicate this discussion, but instead focus on how molecular designs can be utilised to increase the safety of a gene drive trial.

Proactive approaches

Proactive designs aim to limit the probability of transgene escape in the first instance. 'Precision' CRISPR-Cas9 gene drives (Esvelt, et al., 2014), which have been demonstrated in yeast (DiCarlo, et al., 2015) target the Cas9 endonuclease to cut a fixed DNA sequence in the genome unique to the specific target population, with the gene drive transgene then copied across into the cut site. The occurrence of such unique targeting sites is more probable in isolated populations derived recently from small numbers of initial founders and therefore may be particularly useful against island invasives. Alternatively, a 'daisy-chain' drive design could be employed (Noble, et al., 2016). Here a CRISPR-Cas9 gene drive is divided into a linear series of sub-components where each component will only drive in the presence of the component directly beneath it in the series. Critically, the basal component in a daisy-chain cannot drive and will be subject to loss over time through purifying selection. These components are then integrated at independent loci in a release strain meaning that the system is constrained spatially and taxonomically (multiple, sequential, components must escape an island population in the same individual or be combined again through interbreeding in order to continue driving) and temporally (selection will erode each basal component of the daisy-chain in turn until it is flushed from the population). Daisy-chain drives are currently being investigated for the island invasives *C. quinquefasciatus* and *M. musculus*, however analysis so far is theoretical, with – to our knowledge – no prototype strains reported in any metazoan.

An alternative proactive approach is to place inherent fitness costs on a gene drive such that it will persist for a time in a target population, potentially suppressing it, but not increase in frequency. Proposed examples include utilising a gene drive to spread a dominant female-lethal transgene, as proposed for mosquitoes (RIDL-with-drive) (Thomas, et al., 2000), and the endogenous *t*-haplotype meiotic-drive system to spread the male-determining *Sry* gene in mice. Although these systems utilise independent technologies and gene targets, their effects are the same: the transgene doubles in frequency each generation but half those individuals inheriting it (females) are non-viable. If transgenic individuals suffer from reduced fitness, or the drive is less than 100% efficient at biasing its inheritance – both of which are likely in the field – these systems will decrease in frequency over time once deployed (Backus & Gross, 2016), reducing the risk of transgene escape from a trial site but also their efficiency as suppression systems.

Responsive approaches

Responsive designs are complete or partial genetic systems, likely themselves gene drives, designed to be deployed in the event of an escaped drive in order to curtail its spread and potentially remove it or its phenotypic effects from a non-target population. These can include for example a 'reversal-drive' designed to target, spread into and disrupt the DNA sequence of an escaped drive, or the 'immunisation-drive' designed to spread into a non-target population and recode the wild-type target locus, making it unrecognisable to an escaped drive (Esvelt, et al., 2014). These designs can be combined into a single 'immunising-reversal' drive and be made less invasive through using the daisy-chain architecture. A more complex 'restoration-drive' design integrates a relatively non-invasive underdominance system (Figs 1 and 2) into this daisy-chain 'immunising-reversal' drive to theoretically allow the entire system to be flushed from the non-target population once the escaped drive has been halted (Min, et al., 2017).

Although reversal drives have been demonstrated in lab yeast colonies (DiCarlo, et al., 2015) and a non-driving equivalent in *Drosophila* (Wu, et al., 2016) it is unclear how effective these and other responsive approaches would be in the field. There is also concern that, in the event of an escaped gene drive, there may be considerable pressure against rectifying the situation through the release of another gene drive. A more realistic, but not mutually exclusive, approach would be to integrate a high level of conventional control methods at all potential transgene escape points (e.g. connected docking areas/airstrips) during and for a period after a trial. It is clear that responsive approaches should not be relied upon as critical containment methods during a gene drive trial.

Case study: Genetic Biocontrol of Invasive Rodents (GBIRd)

The Genetic Biocontrol of Invasive Rodents (GBIRd) programme aims to develop multiple gene drive systems in mice (*Mus musculus*) for simultaneous evaluation of their safety and efficacy using biosafety standards beyond those required by existing law, while carefully assessing the social, cultural and policy acceptability of such an approach (Campbell et al., 2019). The programme's first stage culminates in the potential submission of an application to a regulatory agency for release of gene drive constructed mice with a spatial control mechanism on a small, biosecure island to test eradication of the wild, invasive mouse population (Campbell et al., 2019). This step-wise approach follows recommendations from USA and Australian National Academies of Sciences (NASEM, 2016; AAS, 2017). Ecological criteria for selecting an appropriate trial island for this application have been proposed (Campbell et al., 2019; Table 1). However, these criteria are just an initial filter and additional steps will be required prior to any potential field trial, including engagement with stakeholders (e.g. land managers, local communities) and regulators to determine final approval (Campbell et al., 2019).

Mus musculus are non-native in countries within the GBIRd partnership (Australia, New Zealand, USA). Mice are not consumed as a food item by people in these countries; negatively impact native species, stored foods, crops, and infrastructure and can carry zoonotic diseases that impact the health of people and their livestock (Stenseth, et al., 2003; Meerburg, et al., 2009; Capizzi, et al., 2014), likely increasing socio-political acceptability. Further, these countries have (or are expected to have) appropriate regulatory capacity and systems established to evaluate a GBIRd proposal, if one is submitted (Campbell et al., 2019). Idealised island selection criteria for potential trials within these countries are provided in Table 1.

CONCLUSION

Gene drives hold enormous potential for application against invasive species and there is increasing interest in adapting them to this purpose. As a transformative but controversial set of technologies, it is important that the first instances of their use in the field are successful, both in terms of efficacy and safety. As discussed, the likelihood of a successful trial can be increased by making appropriate decisions at multiple stages of a gene drive's development and deployment. Making these decisions requires input from a broad range of scientific disciplines (Gould, 2008; Piaggio, et al., 2017) involving, for example, conservationists identifying potential targets, ecologists advising on the biological appropriateness of these targets and efficiencies of different gene drive strategies, molecular biologists advising on the feasibility of building proposed designs, mathematical modellers devising the most efficient means of deploying these systems and, finally, managers who will ultimately advise on the logistic feasibility of deployment. Although described in a linear series, in practice this will require informed dialogue between all these parties from the outset – there is no point in developing a system that performs well in computer models or in the lab if it is ultimately deemed impractical to deploy in the field. With proof-of-principle suppression (Hammond, et al., 2016) and replacement (Gantz, et al., 2015) drives functional in anopheline mosquitoes, it is critical that these conversations begin now to ensure these technologies are applied as safely, efficiently and rapidly as possible to the control of invasive species.

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