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Global Ecology and Conservation

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Review Paper

Identifying knowledge gaps for gene drive research to control invasive animal species: The next CRISPR step

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ARTICLE INFO

Article history:

Received 22 September 2017

Received in revised form 21 December 2017

Accepted 21 December 2017

Available online xxx

Keywords:

Invasive species

Gene drive

CRISPR

Pest management

Islands

ABSTRACT

Invasive animals have been linked to the extinctions of native wildlife, and to significant agricultural financial losses or impacts. Current approaches to control invasive species require ongoing resources and management over large geographic scales, and often result in the short-term suppression of populations. New and innovative approaches are warranted. Recently, the RNA guided gene drive system based on CRISPR/Cas9 is being proposed as a potential gene editing tool that could be used by wildlife managers as a non-lethal addition or alternative to help reduce pest animal populations. While regulatory control and social acceptance are crucial issues that must be addressed, there is an opportunity now to identify the knowledge and research gaps that exist for some important invasive species. Here we systematically determine the knowledge gaps for pest species for which gene drives could potentially be applied. We apply a conceptual ecological risk framework within the gene drive context within an Australian environment to identify key requirements for undertaking work on seven exemplar invasive species in Australia. This framework allows an evaluation of the potential research on an invasive species of interest and within a gene drive and risk context. We consider the currently available biological, genetic and ecological information for the house mouse, European red fox, feral cat, European rabbit, cane toad, black rat and European starling to evaluate knowledge gaps and identify candidate species for future research. We discuss these findings in the context of future thematic areas of research worth pursuing in preparation for a more formal assessment of the use of gene drives as a novel strategy for the control of these and other invasive species.

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1. Introduction

Globally, the pressures that invasive animal species place on biodiversity and agriculture are clearly recognised (Bellard et al., 2016; Doherty et al., 2016). These impacts are exaggerated in landscapes such as islands, including large island

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nations like Australia and New Zealand, where introduced species that became invasive are now linked to the localised or widespread declines or extinctions of native wildlife, and to large economic losses (e.g. [Medina et al., 2011](#); [Cox et al., 2013](#)). Islands are particularly important assets for many countries yet remain vulnerable to introduced species that are having a disproportionately large effect on island ecosystems: for example, islands make up 5% of the global landmass and host some 19% of bird species and 17% of rodent species, yet 61% of all extinct species and 37% of critically endangered species remain confined to islands ([Tershy et al., 2015](#)). New Zealand, for example, is now on an ambitious path to eradicate several introduced predators that are major threats to their native wildlife ([Russell et al., 2015](#)).

As an island nation, Australia has a vested interest in the management of invasive species that impact on both native fauna conservation and agricultural production. For example, predation by feral cats and the European red fox have collectively been scaled as the highest contributing threats in the decline of Australia's terrestrial mammal fauna ([Woinarski et al., 2015](#)). Further, an average \$A620 million over 5-years is the estimated impact on agriculture by pest animals ([Gong et al., 2009](#)). Control techniques typically consist of integrated chemical and physical management practices (e.g. poison baiting combined with habitat removal, fencing), direct intervention (e.g. shooting, trapping), and biological control (for rabbits). While current efforts to manage invasive species are ongoing, these have varying levels of efficacy at low population densities, are often associated with undesirable welfare outcomes for target and non-target species, they may also have unintended ecological consequences, and are often limited to short-term results in population control ([Fancourt et al., 2015](#); [Doherty et al., 2016](#); [Kinnear et al., 2016](#)). These constraints are motivating scientists to look at alternative approaches that are more targeted, economical to implement, reduce animal welfare concerns, and are self-sustaining across large geographical scales ([Campbell et al., 2015b](#)). Practices that extend control to the landscape scale are particularly warranted for invasive species with wide distributions.

Alternatives to conventional control for reducing the population size of some invasive species have been considered ([Campbell et al., 2015a](#)). For example, techniques that aim to introduce sterile males into populations and across generations have been successfully used to reduce invertebrate pests ([Dyck et al., 2005](#)), and have been proposed for cane toads ([Koopman, 2006](#)) and carp ([Gutierrez and Teem, 2006](#)). However, these alternatives require massive releases of sterile males that models predict are labour-intensive and likely have little sustainable impact. It is also a challenge to explain to the public and other stakeholders why large numbers of an invasive pest are being released into a control area. Gene drives are now being discussed as tools to reduce invasive animal populations ([Burt, 2003](#); [Dearden et al., 2017](#)). These genetic elements produce a biased form of inheritance and can spread through sexually-reproducing populations at a greater rate than genes with standard Mendelian inheritance ([Esvelt et al., 2014](#)). While ambitious and still in early stages of development, the gene drive concept offers a species-specific and potentially non-lethal alternative to conventional methods and other forms of genetic control for managing invasive species ([Harvey-Samuel et al., 2017](#)).

A genome editing technique known as Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) and the associated nuclease, CRISPR-associated protein 9 (Cas9) has been adapted to create a "driving mechanism" for a gene cassette to increase the chances of the cassette being passed on to the offspring ([Esvelt et al., 2014](#); [Gantz et al., 2015](#)). This form of synthetic RNA-guided gene drive is actively being discussed as a tool to drive a genetic trait into a wild population ([Thresher et al., 2014](#); [Campbell et al., 2015a](#); [Johnson et al., 2016](#)). The CRISPR gene editing tool uses a nuclease, Cas9, that is directed with a high degree of specificity to cleave a target DNA sequence, and thus can be used to either disrupt, remove, edit, or insert genetic traits in an organisms' genome with great precision ([Doudna and Charpentier, 2014](#); [Shigeta et al., 2016](#)). Evidence from laboratory trials suggests that RNA guided gene drives based on the CRISPR/Cas9 system could spread a targeted gene through nearly 100% of individuals in populations of yeast, fruit flies, and mosquitoes ([DiCarlo et al., 2015](#); [Gantz et al., 2015](#); [Hammond et al., 2016](#)). While the functionality of the CRISPR/Cas9 gene editing tool remains limited to some taxa, there is now strong interest in developing this technology in the laboratory, and ultimately in field trials, for vertebrates that are pests in animal agriculture and conservation ([Ni et al., 2014](#); [Webber et al., 2015](#)). Although offering great potential, this opportunity requires considered planning of the biological and social risks and barriers ([Dearden et al., 2017](#)), and to evaluate which invasive species may be appropriate for initial proof-of-concept efforts and to determine an appropriate approach to risk assessment.

RNA guided gene drives could be used to introduce a number of different gene traits to directly control the numbers of a pest species, or to reduce an environmental impact of a pest (e.g. interfering with the transmission of a targeted biological characteristic, or blocking a toxin-producing gene, [Tingley et al., 2017](#)). However, one application that is gaining interest for many pest species is the disruption of the sex-determination process to bias inheritance of one sex over another across generations leading to skewed sex ratios, decreased fertility and fecundity, and ultimately population suppression or extirpation ([Deredec et al., 2008](#); [Alphey, 2014](#); [Esvelt et al., 2014](#); [Gantz and Bier, 2015](#)). Male transgenic progeny carrying an additional copy of the *SRY* gene (male phenotype control gene) on a gene drive on an autosomal chromosome will father offspring all of which will carry *SRY* regardless of their sex chromosome complement, and will all develop as phenotypic males, all of who will in turn father only male offspring, reducing the number of fertile females until few or none remain in the population. This concept becomes an attractive (albeit theoretical) consideration for sustainable landscape-level control programs. Although much of the established theory on genetically-assisted population suppression has been developed around the requirements of insects (e.g. [Dowling et al., 2015](#)), *SRY*-gene drives have been proposed as a promising approach for the control of invasive vertebrate pests whose impact is primarily direct interference with native species or agriculture ([Burt, 2003](#)).

RNA guided gene drives as a concept for pest animal control are appealing as they are contained within species boundaries by virtue of the fact they are delivered and disseminated by sexual reproduction requiring that it generates fertile offspring (the definition of a species boundary). This enables widespread delivery of a control mechanism (across landscape scales) and eliminates off-species-target effects. While these gene drives in their existing form are also highly invasive there is much current research into self-regulating gene drives that provide greater control and present less risk to nontarget populations. Australia has a unique environment in which to consider such a control strategy since there is a large evolutionary distance between Australian native species and the invasive animal species that are impacting on its ecosystems.

The application of gene drive technology for invasive species control needs to consider the level of knowledge currently available on population genetics and ecology that can be used as a basis for undertaking the necessary ecological risk assessments and trials. Gaps in knowledge will hinder the future progress of gene drive work on invasive species. The opportunity therefore exists in Australia to understand where these knowledge gaps lie and how they will impact on the decision whether or not to consider gene drive as a control for a particular species of invasive animal. Here, we review the current biological, genetic and project logistics knowledge related to invasive species within the Australian context and relevant to gene drive research, we evaluate gaps in knowledge, and propose strategies to address these gaps to aid future research practices. This work has been motivated by the current (and dramatic) level of impact many invasive species have on Australian ecosystems and agriculture.

2. Methods

2.1. Conceptual model

Within the context of an ecological risk framework (Landis, 2003; National Academies of Sciences, Engineering, and Medicine, 2016), we have developed a modified conceptual model. This model illustrates the relationships between an invasive species targeted for gene drive research in the Australian context, and a suite of biological, environmental and logistical parameters (stressors) that may influence future research on the species, or that may affect survival and release of a modified organism into the environment. This framework identifies factors contributing to potential risks related to the persistence and spread of a gene drive organism. The intent is to address knowledge gaps to reduce uncertainty in relation to these risks. The model focuses on four interconnected nodes: source and genomic information of a target species, effect of releasing a transgenic species on conspecifics, effect of releasing a transgenic species into the environment, and outcome (project goal).

2.2. Gap analysis and evaluation

We used the risk model to conduct a gap analysis to signpost future needs for gene drive (using the CRISPR-Cas9 nuclease system) research in an invasive species. We applied this framework to seven exemplar invasive species in Australia representing amphibian, avian and mammalian taxa, based on their acknowledged impacts to biodiversity and agriculture: cane toad (*Rhinella marina*), European starling (*Sturnus vulgaris*), house mouse (*Mus domesticus*), European red fox (*Vulpes vulpes*), feral cat (*Felis catus*), European rabbit (*Oryctolagus cuniculus*) and the black rat (*Rattus rattus*). We identified potential biological, environmental and logistical factors that may influence future research opportunities, or that may affect individual survival in the receiving environment. We conducted a literature review to summarise this information for each species into available knowledge and desirable biological and logistical requirements (based on this knowledge) in the context of the conceptual model and gene drive research. We then applied a qualitative scoring system to compare and evaluate each species as a candidate for the future research of gene drive technology. Knowledge available for each species was assessed by the authors to be lacking (score 0), minimal (score 1), or relatively good (score 2). Similarly, desirable requirements in each species were scored as not (score 0), partially (score 1) or fully (score 2) present or documented. We refrained from weighting each character as we intend the scoring to be equally relative to other features scored, and accept this could be a further refinement approach to our evaluation. Finally, we review this information to identify gaps in knowledge, how these may be addressed, and the implications for future gene drive work when considering these candidate species.

2.3. Exemplar species

House mice were likely introduced to Australia from Western Europe, predominantly Britain (Gabriel et al., 2011). House mice are now ubiquitous across urban, agricultural and natural landscapes in Australia. In the wheatbelt of southern and eastern Australia house mice occasionally erupt into rapid population expansions, often referred to as 'mouse plagues'. These outbreaks cause significant damage to crops, place rural communities under great stress due to financial impact and frequent direct encounters, pose a potential health risk and are an environmental threat (Singleton and Redhead, 1989). In Australia, mice are vectors of a variety of infectious diseases that may be transmitted to humans and other livestock (Caughley et al., 1998). Mice can transmit salmonella to one another, to humans and to domestic animals; encephalomyocarditis virus to pigs; fungal skin diseases (ringworms) to cats and humans; and leptospirosis to humans and domestic pigs. Control of mice is usually achieved by trapping and poisoning which achieves a degree of short term success. However, kill-traps are labour intensive to maintain and do not discriminate between target rodents and non-target animals. Also, rodenticides (e.g.

Brodifacoum and zinc phosphide) and kill-trapping methods have associated animal welfare (target and non-target), human health and safety issues. Aerial baiting programs for house mice on islands have had some success (e.g. Howald et al., 2007); however, this approach is expensive. Modelling of alternative biocontrol methods involving rodent specific parasites suggest limited potential for this approach (McCallum and Singleton, 1989).

Feral European rabbits were first reported in Tasmania from the early 1800s, and on continental Australia there were some 12 documented releases in 1859 (Rolls, 1969). By 1910, they covered most of their present range, which represents close to two thirds of Australia. They are both a chronic pest to agriculture - with a current economic loss of some A\$200 million annually (Cooke et al., 2013) - and to the natural environment where they damage vegetation, degrade the land, are an important prey item for introduced foxes and feral cats, and compete for resources with native fauna (Pech and Hood, 1998). Competition and land degradation by European rabbits are listed as a Key Threatening Process under Australia's Commonwealth legislation (*Environment Protection and Biodiversity Conservation* [EPBC] Act, 1999). Their control is complex because rabbits are a food item for introduced and invasive predators (fox, wild dog, feral cat), and there are community expectations that animal welfare issues need to be considered during their control. Current control strategies combine integrated biological, chemical and mechanical approaches. In relation to rabbit biocontrol, effectiveness is patchy both spatially and temporally (e.g. Richardson et al., 2007; Liu et al., 2014; Wells et al., 2016) and many individuals, and subsequently populations, exhibit varying degrees of resistance to virus strains (e.g. Robinson et al. 2002; Mutze et al., 2010a) which has enabled rabbit numbers to increase over time (Cox et al., 2013). Importantly, biological control must be combined with conventional techniques in order to be effective (e.g. McPhee and Butler, 2010; Mutze et al., 2010b).

The European red fox is native to Europe, Asia, North America, and North Africa. It was deliberately introduced into eastern Australia in the 1870s by acclimatisation societies (Rolls, 1969), and has rapidly spread across the continent following the spread of the rabbit (Dickman, 1996a). Predation by foxes has been directly linked to the loss of mammal fauna across the continent, in particular, small and medium-sized mammal species (Kinnear et al., 2002; Saunders et al., 2010; Woinarski et al., 2014), and foxes are a serious agricultural pest (McLeod, 2004). Foxes are recognised by the Australian Government as a Key Threatening Process under the EPBC Act (1999). Current control techniques include trapping, shooting, poisoning with 1080 (sodium monofluoroacetate), den fumigation, and exclusion fencing (Saunders et al., 1995). While locally effective, these methods require ongoing resources and management effort and, in some instances, the effect of baiting shows mixed results (Kinnear et al., 2016).

The feral cat became established in Australia since European settlement (Abbott, 2002). Cats are suggested to have spread over the continent from multiple coastal introductions after the 1820s. Feral populations became established around settlements and spread from there, and cats were also transported to many locations as a means of controlling rabbits, mice or native species (Abbott, 2008). By 1890 most of continental Australia had been colonised (Abbott, 2002). Predation by feral cats is listed as a Key Threatening Process under the EPBC Act 1999. Feral cats pose particular problems because they are difficult to control. They are also the sole host and vector of the protozoan parasite *Toxoplasmosis gondii* that affects some native species and also domestic cats (Bettiol et al., 2000; Fancourt et al., 2015). Similar to foxes, the direct impacts of feral cats on Australia's fauna (particularly species in the critical weight range 35–5500 g) is mounting (Gibson et al., 1994; Christensen and Burrows, 1995; McKenzie et al., 2007; Hardman et al., 2016). They have caused the decline and extinction of native fauna on islands as well as contributing to a significant impact on ground birds and small native mammals (Dickman, 1996b; Risbey et al., 1999; Doherty et al., 2016). Current management options for feral cats include exclusion (eradication within a fenced area) and integrated lethal control (e.g. poison baiting, shooting, trapping); however, the later are temporary solutions requiring ongoing control practices. Baiting for feral cats has been shown to be ineffective during times when food resources are abundant and cats have ready access to preferred sources of food (Short et al., 1997; Algar et al., 2002; Christensen et al., 2013).

Cane toads were deliberately introduced from Hawaii to Australia in 1935 to control scarab beetles that were pests of sugar cane. They are listed as a Key Threatening Process under the EPBC Act, and by the World Conservation Union as one of the world's worst 100 invasive alien species. Cane toads have now spread through much of tropical and subtropical Australia (Sabath et al., 1981; Phillips et al., 2007). Few control methods currently exist. The cane toad is a highly invasive species, and has poison glands that are toxic to most native mammals, reptiles and other taxa (Phillips et al., 2003; Doody et al. 2009; Woinarski et al., 2009). As a result, their spread across northern Australia is linked to declines in several native species: native frogs (Murray and Hose, 2005), northern quolls, *Dasyurus hallucatus* (Burnett, 1997), geckos (Watson and Woinarski, 2003), and beetles (Catling et al., 1999). Despite localised and labour-intensive 'toad buster' control efforts by volunteer groups to capture cane toads by hand, cane toads continue to move westward across the Kimberley region of Western Australia and it is now clear that this movement cannot be stopped using any of the methods currently available (Department of Parks and Wildlife, 2014).

The black rat is believed to have been unintentionally introduced into Australia sometime during early settlement by Europeans (Long, 2003). It is well known globally for its negative effect on a number of species, particularly seabirds on islands (Jones et al., 2008). In Australia, when established, they are known to outcompete native rodent species (Banks and Smith, 2015), and to negatively influence seabird colonies (Townes et al., 2009; Banks and Hughes, 2012). Predation by this species (in addition to other exotic rats) on offshore islands is a Key Threatening Process under Australian legislation. Contemporary control measures (and eradication programs on islands) at the landscape scale typically involve aerial broadcast of rodenticides (Howald et al., 2007), and belie similar issues to the control of house mice in terms of non-target impacts.

European starlings were released in capital cities in southeastern Australia by acclimatisation societies in the mid-19th century (Rolls, 1969). Starlings occupied much of the southeast of the continent including Tasmania by the turn of the 20th century, and incursions to the west of the country have been regularly reported along the south coast since the 1970s (Woolnough et al., 2005). These western populations are largely contained due to an on-going control program including live trapping and shooting, supplemented by netting (cannon and mist) and strategic poisoning (Campbell et al., 2015b). Current control efforts in Western Australia require ongoing surveillance and shooting to keep numbers down (Woolnough et al., 2005). If left, these western populations have a high probability of expanding (Campbell et al., 2015b). Recent genetic evidence suggests there are unknown sources of founder birds migrating to the western part of the continent (Rollins et al., 2011).

3. Results

Our review of the literature identified 10 biological and two project logistical requirements that provide useful information within the context of the conceptual model (Fig. 1, Table 1). The source node includes what we know about the distribution and suitable habitat of a species, the characteristics currently available for a reference genome, and knowledge about the capacity to contain, rear and maintain a breeding colony in captivity (animal husbandry).

The second node focuses on the risks associated with the spread and persistence of a transgenic animal that may be influenced by individual and population-level stressors that act to alter reproduction, survival and dispersal: breeding seasons, mating systems and reproductive biology, which may influence post-release gene flow in target populations. Knowledge of the spatial ecology of a species helps to identify population structure and potential barriers to breeding and gene flow, either or both of which can slow the spread of a gene drive through a population. Additional stressors - driven by climate and resource availability, the presence of existing biocontrols, or translocation stress in the presence of established conspecifics - may regulate the dynamics and survivorship of individuals at the population scale.

The third node focuses on the effect of a transgenic animal once released into the receiving environment. Risks may be evaluated in terms of negative changes to community processes (e.g. mesopredator release) that may occur when a targeted invasive species is removed, the unintentional transfer of genes to non-target species through interspecific breeding (horizontal gene transfer), and to intraspecific breeding with conspecifics in their native range outside Australia (implicitly border security requiring information related to the unintentional or intentional movement of species from Australia). While sexual reproduction is a fundamental characteristic needed for the transfer of a gene drive between a transgenic population and targeted wild type population, we have not considered this further in the model because all our exemplar invasive vertebrate species satisfy this requirement.

Finally, the outcome node reflects the desired management goal: in our example scenario this relates to invasive species population control by biasing offspring sex ratios, although other gene drive strategies may define alternative end goals.

Rabbits and house mice scored highest in our assessment of available knowledge (22 and 21, respectively, out of a total score of 24; Table 2, also see Tables A1, A5). Relevant knowledge currently available for fox, cane toad and feral cat is also well established in the literature, although fox scored marginally higher primarily because there are studies in wild animals that have also investigated their fertility control (Table A2). Importantly, research knowledge exists on the sex-determining

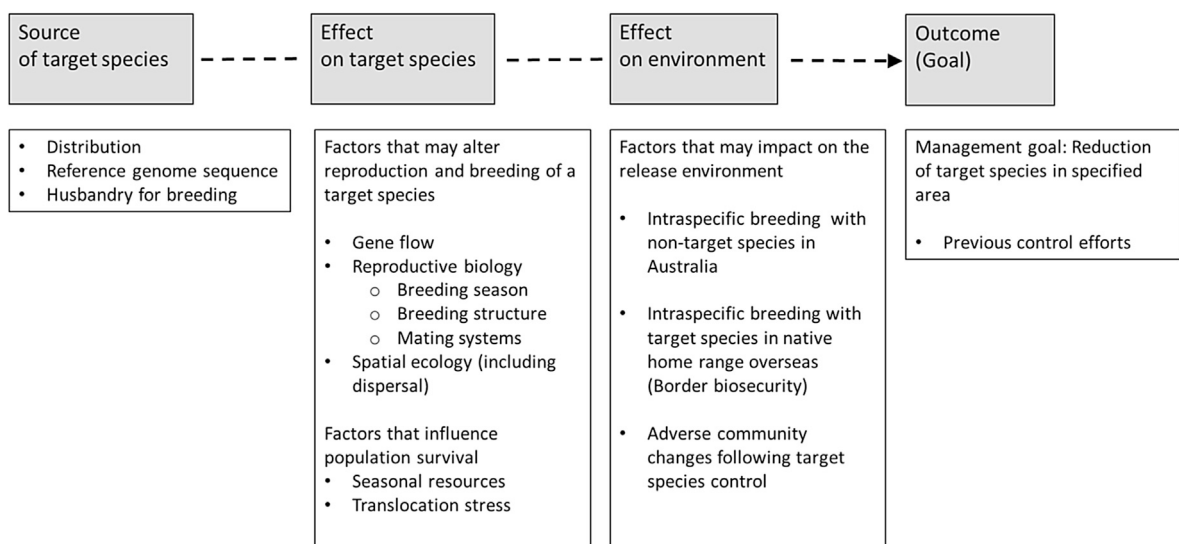


Fig. 1. Conceptual model to illustrate the four key nodes (shaded) and types of information (unshaded) required to reduce gaps and uncertainty about the spread and persistence of a gene drive construct focusing on invasive species control as the management goal.

Table 1

Desirable biological requirements and logistical parameters of invasive species when considering a gene-drive investigation. In our assessment, we consider gene drives to bias population sex ratios.

Conceptual model node	Requirement	Explanation	Desirable features of invasive species for a gene-drive investigation
Source			
	Distribution	The geographical spread of an invasive species can assist managers to decide whether population control (where there is no population containment) or eradication (population containment) is the feasible goal. Physical geographic boundaries (such as islands) are available at a range of scales which may help manage the spread of transgene release beyond the area of interest.	<ul style="list-style-type: none"> Species with localised disjunct populations to enable managed control or eradication (e.g., island populations exist at a range of scales (distance to coast, area)).
	Genome sequence	A reference genome sequence is critical for gene drive research that targets genes for a particular trait. For example, male sex determining genes are the focus of gene drives to reduce (bias) female births in a population. For this type of control action, identifying and sequencing sex-determining genes, and known molecular links between the target gene and other genes will be important for CRISPR/Cas9 specificity. Alternative approaches to modify genes (e.g. blocking production of a toxin, controlling growth) will necessitate a knowledge of other target genes.	<ul style="list-style-type: none"> Sequence of target gene
	Husbandry and transgenic systems	Understanding whether an invasive species can be reared and contained as breeding colonies in captivity at an appropriate scale – important for conducting experimental trials – is a necessary precedent for gene-drive research for embryology and reproductive studies. Further, development of transgenic systems is required to introduce gene editing into animals.	<ul style="list-style-type: none"> Species has been successfully reared, bred, and contained in captivity. Gene constructs can be introduced to animals.
Effect on species			
	Gene flow	Gene flow in a population can vary based on the stage of the life cycle (passive dispersal of eggs vs individual dispersal into new habitats), type of movement (whole organism vs gametes), and the geographic scale of movement. Under contained conditions, random mating and high gene flow will ensure a gene drive passes through a target population efficiently. This is useful information for risk assessments that deal with the likelihood of transfer of genetic material to non-target organisms or populations. Models to simulate post-release gene flow in target species can predict the spread of a gene drive through a population under various environmental scenarios, and population parameters (expansion, contraction). These models can predict whether localised control using gene drives is effective.	<ul style="list-style-type: none"> High gene flow in a population. Random mating.
	Reproductive biology and mating system	Gene drives are transmitted by sexual reproduction. The effectiveness of this transmission will depend on the reproductive characteristics of a species (i.e.) fecundity, mate choice that could bias inherited genes, number of progeny contributed to the next generation, mating system (monogamy versus polygamy), and generation time. A gene drive will spread quickly in a population for fast-reproducing species with minimal mate selection and a polygamous mating system, but relatively slowly in longer-lived species.	<ul style="list-style-type: none"> High fecundity. Polygamous mating system. Minimal mate selection Large number of offspring. Short generation time.
	Spatial ecology	The spatial heterogeneity of individuals, including whether dispersal is biased to a demographic group or sex, are vital biological parameters to understand which demographic group and/or sex should be targeted for gene drive research. Gene exchange will be lower in species that disperse rarely or maintain social hierarchies that influence breeding performance compared to species with high dispersal behaviours in their life history. Sex-biased dispersal of the sex carrying the gene drive becomes advantageous for its transmission through the population.	<ul style="list-style-type: none"> Species with a high overlap in home range areas. Sex-biased dispersal (for sex-determining gene drives).
	Population regulation	One challenge with managing invasive species stems from a lack of information regarding the drivers of their population dynamics. The transmission of gene drives will be self-limiting or self-sustaining based on a variety of stressors regulating the population over time (biological, ecological climatic). These stressors may each exert an influence on the survivorship of offspring, and the subsequent transfer of genes between generations. Models to simulate population growth rates can	<ul style="list-style-type: none"> Understanding the drivers of population dynamics including growth rate, age structure, and density dependent population growth.

Table 1 (continued)

Conceptual model node	Requirement	Explanation	Desirable features of invasive species for a gene-drive investigation
	Translocation stress	<p>assess the spread of a gene drive through a population under various environmental perturbations, and population parameters (expansion, contraction).</p> <p>Little is known about the mechanisms contributing to the post-release survival of many invasive species released into existing populations of conspecifics because research often focuses on releases into novel environments (e.g. Nathan et al., 2015). Understanding how translocation affects the survival and successful establishment and dispersal of initial gene drive colonists into a population of conspecifics provides important information on the population processes driving biological invasion and establishment, and the future transmission of genes through a population.</p>	<ul style="list-style-type: none"> Species with high survivorship and reproduction following release into habitats with conspecifics.
Effect on environment	Intraspecific breeding	The presence of related, sexually-compatible species in an environment is of relevance where targeted control using gene drives is sought. Evidence of sexual reproduction of a target species with a related species represents a risk for the transmission of a gene-drive to non-target species (horizontal gene transfer). The consequences could lead to the suppression or extinction of a non-target species.	<ul style="list-style-type: none"> No interspecific breeding.
	Border biosecurity	The development of a gene-drive organism must include safeguards to mitigate the risk of transgenic species moving to other locations or beyond Australia to their native country of origin where they have conservation value (Oye et al., 2014). Biosecurity risks may be particularly high for species associated with human movement and trade which stowaway among cargo and trade goods that leave Australia, or species which show a propensity/capacity for long-distance migration or rafting.	<ul style="list-style-type: none"> No - or remote – likelihood of transportation of an invasive species outside of Australia. Proven containment pathways for species of interest.
	Adverse community change	The local extirpation of invasive species may have implications to ecosystem food webs (Zavaleta et al., 2001). For example, some invasive species can suppress populations of other invasive predators that would then otherwise impact on endemic species (Howald et al., 2007; Molsher et al., 2017). In such cases, removal of one species can have cascading trophic effects on the distribution and abundance of others through processes such as mesopredator or prey release.	<ul style="list-style-type: none"> No - or minimal negative - change to the abundance of non-target invasive species following a reduction in abundance of the target species. Increase in activity and/or abundance of native species has been demonstrated following the control of the target invasive species.
Endpoint	Control	Gene drives may be used for a variety of purposes (e.g. Esvelt et al., 2014). For gene drive research investigating sex bias, a species where fertility control has previously been demonstrated, and where information is available on reproductive and behavioural performance and output, will be an advantage if future gene drive work is to focus on sex-biased population suppression. Information on other genes will be useful for the development of daisy-chains or other self-limiting gene drives.	<ul style="list-style-type: none"> Previous control efforts have been demonstrated in the target species. For sex-biasing gene drives, fertility control has been demonstrated in target species.

gene(s) for each of the selected mammals; however, this information is in early development for black rats and starling. Although cane toads score high in total available knowledge relative to our other exemplar species, there are gaps in terms of a sequenced and annotated genome (presently under investigation) as well as a lack of data on the role the various sex-determining genes play during male development (Table A4).

Mate choice plays a role in reproductive performance in house mice, rabbits, cane toads and starlings; however, this information is lacking for foxes, feral cats, and black rats. At the population scale gaps in our current knowledge relate to gene flow between and within fox or black rat populations, mating systems in feral cats (that lead a primarily solitary habit within the Australian context), spatial structure and drivers of population change in starlings, and realising whether there may be adverse changes in the abundance of other pest species in a community with the removal of cane toads or starlings.

While all the selected species are known, credible and successful invaders of habitats (i.e. generalists, adaptable to new environments), we found no invasion biology data about the survivorship or reproductive potential of individual foxes, feral cats or black rats if introduced into existing landscapes with conspecifics. However, there is knowledge on some species when introduced into areas with conspecifics: survivorship for house mice on islands, rabbits moved between warrens within a European context, cane toads transported between locations, and starlings at the western invasion front of their range.

Table 2

Evaluation scoring of available knowledge of desirable requirements (lowest value reflects highest gap) for exemplar invasive species within the Australian context, or informed by life-history characteristics derived from data elsewhere. Total values are cumulative. Knowledge about the species was assessed by the authors to be lacking (score 0), minimal (score 1), or relatively good (score 2).

Requirement (Knowledge)		European rabbit	House mouse	Invasive species				
				Red fox	Cane toad	Feral cat	European starling	Black rat
Source	Distribution	2	2	2	2	2	2	2
	Molecular biology	2	2	2	1	2	1	1
	Husbandry	2	2	2	2	1	2	2
Effect on species	Gene flow	2	2	1	2	2	2	1
	Reproductive biology and mating system	2	2	2	2	1	2	2
	Spatial structure	2	2	2	2	2	2	0
	Population regulation	2	2	2	2	2	1	0
	Translocation biology ^a	1	1	0	2	0	1	0
Effect on environment	Intraspecific breeding	2	2	2	2	1	2	1
	Biosecurity	1	0	1	2	1	1	0
	Community change ^b	2	2	2	0	2	0	1
Endpoint	Fertility control	2	2	2	0	1	0	0
TOTAL		22	21	20	19	17	16	10

^a Translocation into existing populations of conspecifics.

^b Negative community changes due to the niche 'release' of other introduced species.

Evidence of the accidental transport of cane toads between areas across Australia, and one record from Australia to New Zealand, suggests a border biosecurity risk associated with cane toads (Table A4). Information on the planned or unplanned exportation of rabbit, fox, starling, feral cat, and black rat from Australia is either minimal, or lacking.

Information to investigate the reproductive output of a species following a level of fertility control (e.g. modelling fertility control under various scenarios, or experiments on the surgical sterilisation of females) has been conducted on rabbits (Table A5) and house mice (Table A1) as part of previous studies investigating the biological control of these species. The results of these experiments showed reproductive compensation occurred in female mice and rabbits whereby fertile individuals reproduced more often and there was an improved survival of juveniles. In a similar vein, models for fertility control and field experiments for the fox suggest that reproductive performance is affected by climate, resources and fox density in the environment (Table A2). Information on fertility control experiments is limited to domestic cats, and does not exist for free-ranging cane toad, black rat and starling.

While a solid level of knowledge may be available for many exemplar invasive species within the Australian context, the desirable features of each species varies with their life history characteristics or with a lack of evidence to support the characteristic sought (Table 3). The house mouse and rabbit scored equally highest (21) in terms of desirable characteristics that would make them suitable gene drive organisms for study. However, house mice rank low from a biosecurity perspective

Table 3

Evaluation scoring based on presence of desirable requirements for invasive species within the Australian context. Total values are cumulative. Desirable requirement not (score 0), partially (score 1) or fully (score 2) present or documented.

Requirement (Present or demonstrated)		House mouse	Invasive species					
			European rabbit	Feral cat	Red fox	Cane toad	European starling	Black rat
Source	Distribution	2	2	2	2	2	1	2
	Molecular biology	2	2	2	2	1	1	0
	Husbandry	2	2	1	1	2	2	2
Effect on species	Gene flow	2	2	2	1	2	2	1
	Reproductive biology and mating system	2	1	1	1	1	1	1
	Spatial structure	2	2	2	2	2	2	0
	Population regulation	2	2	2	1	2	1	1
	Translocation biology ^a	1	2	0	0	1	1	0
Effect on environment	Intraspecific breeding	2	1	1	2	1	2	1
	Biosecurity	0	2	2	1	0	1	0
	Community change ^b	2	1	1	1	0	0	0
Endpoint	Fertility control	2	2	1	1	0	0	0
TOTAL		21	21	17	15	14	14	8

^a Translocation into existing populations of conspecifics.

^b Negative community changes due to the niche 'release' of other introduced species.

because of their commensal habits around ports and their propensity to stowaway with cargo. Information about the invasion biology and survival of individual mice when introduced into extant social groups is limited, a characteristic critical to understanding the breeding performance of transgenic mice introduced into new areas with existing mice. Rabbit scores are low for reproductive performance (colonies have a social structure that limits breeding to dominant pairs), and intraspecific breeding due to the potential of transgenic rabbits mating with domestic rabbits. While rabbits score low for adverse community change brought about from the potential of introduced predators to prey-switch to native species when rabbit densities are low, this change is short-term before predator densities also crash with reduced food in the landscape.

As key invasive predators of Australian landscapes, foxes and feral cats also rank well in terms of desirable features for supporting future transgenic research, but there are gaps. In terms of desirable reproductive features, fox and feral cat score low because of the length of time juveniles take to reach sexual maturity (10 and eight months, respectively). A primarily monogamous pairing in foxes during the female oestrus period, together with a social dominance in groups and reproductive compensation that influences reproductive output, also contribute to a lower scoring of desirable features for this species. Evidence suggests that the removal of either fox or feral cat from an ecological system must consider mitigations for subsequent trophic community changes in the activity or abundance of co-existing invasive predators and/or invasive prey (Table A2, Table A3). A more probable risk is associated with intraspecific breeding between feral cats with domesticated conspecifics, although genetic evidence suggests this is unlikely and of low consequence (Table A3). Finally, there is a low score, in terms of invasion biology, for foxes or feral cats translocated into landscapes with existing conspecifics. Maintaining breeding colonies of fox and feral cat are also challenging due to the size and primarily solitary habits of these species, and as a means of overcoming domestication. Reports find that reproductive suppression due to dominance hierarchies may occur among captive foxes (Table A2), and feral cats are problematic to maintain as they are a solitary-living species whose physiology and behaviours have been shown to change in captivity (Table A3).

Cane toads and starlings exhibit a number of desirable features although overall they score lower than the other exemplar invasive species. Research is underway to produce a full genome of the cane toad and European starling, although this information is currently not publicly available. Cane toads and starlings may take up to 24 months to become sexually mature. Despite producing several thousand eggs per clutch, only an estimated 1% of cane toads reach adulthood so the likelihood of a gene drive passing to the next generation is not as high as it might at first appear. Some work shows that cane toads and starlings, when artificially moved or naturally dispersed into existing populations of conspecifics, do survive and breed although more work could be conducted on their invasion biology. No information exists on the community-level changes to other invasive species with the removal of starlings or cane toads, so we could not score these features.

Surprisingly, black rats in the Australian context scored lowest. Multiple copies of male-determining genes are known to exist in the genome of the brown rat (*Rattus norvegicus*, Table A6); however, this has not been confirmed in the black rat. There is very little gene flow and ecological data for black rats in the wild in Australia, resulting in lower scores for these desirable features. Some data exist to indicate that the persistence of black rats in Australian habitats may be regulated by the presence or absence of native rats, but this information is limited. Whether black rats establish and breed if placed into habitats with conspecifics also remains unknown. No evidence exists for the community-level changes to other invasive species following the removal of black rats from an area. As with house mice, managing biosecurity for black rats will be challenging for this commensal species. There are several species of native rats in Australia under the *Rattus* genus although no evidence exists of interbreeding with black rats. These possibilities require some consideration to determine the possibility of gene transfer by cross species hybridisation or other indirect impacts to non-target species.

All of our exemplar invasive species experience current control efforts to manage their densities at local (house mouse, cane toad, black rat) or regional (rabbit, fox, feral cat, starling) scales, and evidence shows these densities can be managed, albeit temporarily. Experiments have demonstrated the population-level effects of influencing reproductive output following fertility control in house mice, rabbits and, to a limited degree, foxes and (domestic) cats. It remains unclear what level of density or reproductive compensation may occur for control programs to manage cane toads or black rats, resulting in low scores in these categories in our analysis (Table 3).

4. Discussion

We have developed a framework which aligns to an environmental risk assessment model suggested in the National Academies of Sciences, Engineering, and Medicine report on gene drives (2016). Our results demonstrate that each of the exemplar invasive species we have considered is variously suited to the potential application of gene drive technology. Though no single species scores highly for all the traits considered important in the gene drive context, the analysis has identified current gaps in knowledge critical for the development of this novel control measure and this may help direct future research. An advantage of our model is drawing together and aligning the biological, molecular, reproductive, ecological and project logistics tools needed to support future risk assessments and stage 1 contained trials. Separating the information into nodes offers a thematic approach to address the risks to both individuals and the receiving environment. We suggest this conceptual model not be used for prescriptive purposes but specifically to highlight the knowledge gaps that need to be filled as a gene drive is considered for particular cases of invasive species control.

There is a reasonable level of literature presently available for the exemplar invasive species in Australia in relation to the assessment characteristics (Appendix tables A1–A7). Based on the literature search and analyses the knowledge gaps have been categorised into eight thematic areas.

4.1. Life history and fecundity data (age-specific and sex-specific)

Life history and fecundity information provide the empirical data for spatial models to predict gene flow at high and low population densities. Within an Australian context, quantitative life history and fecundity data currently exists for fox, rabbit, cane toad and house mouse (Estoup et al., 2004; Marlow et al., 2000; McIlroy et al., 2001; Singleton et al., 2001). The high fecundity of cane toads, in particular, makes them suitable during the development stage of gene drives. However, there are significant knowledge gaps in this area for feral cat, black rat, and starling. These gaps make risk assessments challenging as it is not possible to model and evaluate the spread and containment of a gene drive for these species. Robust starling population growth models have been developed for the western invasion front in Australia, informed by life-history characteristics derived from Australia and overseas data (Campbell et al., 2015b). It may be possible to use international literature help to inform models for other species that require life-history information.

4.2. Reference genome

A reference genome is critical for gene drive research, although production of reference genes is becoming routine so this is not the limiting factor that it once was. For investigations of gene drives that focus on sex determination, information exists for the male sex-determining genes in mammals, and assembly of this sequence information is in progress for cane toad, and for starlings (L. Rollins, personal communication). Exploring the functional role(s) of sex-determining genes, including the homing rates of gene drives, will help to predict the spread of these alleles through populations and will be the key to designing efficient and specific gene drive components. For example, modelling the efficacy of a sex-reversing gene drive construct on an island population of rodents and rabbits showed that it failed to persist and that alternative strategies need to be investigated to improve the probability of their eradication (Prowse et al., 2017). Within the context of a male-biasing gene drive, there are gaps in the molecular knowledge for the sex-determining genes of cane toads, black rats and starlings. However, for all our exemplar species, specific gaps remain that relate to the homing process of gene drives, and to estimates of mutation rates producing resistance alleles in a target population.

4.3. Gene flow

Models are required to predict the spread of an RNA guided gene drive construct through a population (Wang et al., 2016). The extent to which transgenic individuals can contribute genes to a resident wild-type population will vary based on the mating dynamics, mate selection, position of a species on the invasion curve, and generation time of target individuals (Esvelt et al., 2014). Time-series stochastic models that map the spread of gene drives and their effects (e.g. sex-ratio distortion) in populations become important for assessing and managing the spread of transmission (Backus and Gross, 2016; Wang et al., 2016; Prowse et al., 2017). There is a gap in the collection of gene flow information for fox and black rat in the Australian context that will be needed to evaluate how gene frequency data, together with life history data, can be incorporated into spatially-explicit models about the likely spread of a gene drive in these populations. Additional information on mate selection will also be needed to understand the rate of spread of a drive between generations for species where a social structure is known to exist (e.g. mouse, fox, rabbit).

4.4. Density dependant reproduction and mate selection

The reproductive biology and mating systems for each species remain critical information for risk assessments: a transgenic animal must be able to effectively spread and reproduce at a rate equal to or greater than wild type animals for a gene construct to be successfully spread through a population and for this biotechnology to be successful. Understanding the drivers of reproduction under varying population densities (e.g. a species' position on the invasion curve, DEDJTR, 2017) provides foundational knowledge to predict the likelihood that a gene drive will persist and spread. Pest populations have been shown to exhibit strong compensatory density-dependence as the population declines due to the increased survival, reproductive output and/or fitness of remaining (unmodified) individuals (Chambers et al., 1999b; Zipkin et al. 2009; Marlow et al., 2016). The results of field trials that simulate the effects of fertility control suggest that compensatory responses to fertility control may range from relatively weak (in the case of the fox, Saunders et al., 2002) to strong (house mice, rabbits, cane toads; Chambers et al., 1999a, 1999b; Twigg and Williams, 1999, Twigg et al., 2000, Williams et al., 2007, Pizzatto and Shine, 2008). A higher fertility at lower densities may be an advantage for gene drive suppression programs if there is no or limited reproductive selection for individuals without the inherited gene drive. Understanding whether mate choice occurs in fox, feral cat, and black rat will alter predictions of gene flow for these species. It remains unknown whether density-dependent reproduction exists for feral cats, black rats and starlings in the Australian context.

4.5. Border biosecurity pathways

Information on the transport pathways and the movement of invasive species across Australian borders is either lacking or is observational for most of the exemplar species. Concerns relating to transgenic species include intraspecific breeding with conspecifics outside of a geographic area or back into native populations, potentially through intentional human action

(Webber et al., 2015). However, Australia, as an island continent and single jurisdiction, is well situated to mitigate transgene escape overseas and able to implement biosecurity risk management for key transport pathways (Williams, 2007). Molecular safe-guards exist to reduce the probability of gene drives spreading elsewhere through hybridisation with conspecifics (Noble et al., 2016) or to overwrite and block the effect of gene drives (e.g. DiCarlo et al., 2015), and further research into self-limiting gene drives is continuing. Data to estimate species movement pathways and analyse the effectiveness of current biosecurity will best inform risk likelihood assessments based on current management practices (Azmi et al., 2015). Destination ports are of particular interest because stowaways may be missed at exit ports, and the number of stowaways exiting Australia alive will likely differ from the number that actually remain alive through to the destination. In terms of desirable characters, border biocontainment of house mice and black rats will be challenging to manage because of their propensity to stowaway and survive among cargo and vessels. Similarly, minimal information exists about the biosecurity pathways related to the unplanned transport of wild rabbits, fox, feral cat and starlings overseas. From a risk perspective, evaluating this pathway information from shipping records and ports around the country can provide valuable knowledge to direct strategic biosecurity risk assessments for intercepting the unplanned movements of each of these exemplar species out of Australia.

Consistent with the recommendations of the NASEM report (2016) one place to trial gene drive technology, where biosecurity risk can be managed with some confidence, is offshore islands. The natural barrier of the sea enables management of the risks associated with the biocontainment of transgenic animals and therefore gene drive constructs. Islands also offer a place where eradication may be viewed as a feasible end-goal. Information on the gene flow between geographically disjunct populations will help to understand the degree of genetic isolation that islands impose, and to evaluate the biocontainment risk (Marsden et al., 2013). Other than starlings, the exemplar species investigated in this study are all ground-dwelling and have island populations where dispersal to the adjacent mainland is minimised by seawater.

4.6. Community interactions

Understanding the community interactions between invasive species and resident pest and native species is essential for undertaking a successful landscape restoration program. While the removal of one invasive species from an area is known to have positive effects on an ecosystem (e.g. Pedler et al., 2016), there are examples where these actions have had no effect or negative, unpredicted effects on the ecosystem as a result of changes among communities of other invasive species (Zavaleta et al., 2001; Howald et al., 2007). These outcomes have often been the result of a lack of knowledge about the interactions between species in those ecosystems. In Australia, community interactions have been demonstrated in ecosystems where foxes coexist with feral cats and rabbits, such that suppressing one invasive species requires concurrent management of the other invasive species (Algar and Smith, 1998; Holden and Mutze, 2002; Marlow et al., 2015). However, control of just a single invasive species may be beneficial to native species as evidence has shown that the local control of house mice resulted in an increase in the numbers of small native small mammals (Dickman, 1992; Moro, 2001). Information about the community and/or trophic changes to native biodiversity following the removal of invasive species is currently emerging for rabbits (Pedler et al., 2016), remains minimal for black rats and starlings (Pell and Tideman, 1997; Banks and Smith, 2015) and has yet to be explored in detail for cane toads. There is a clear need to better understand the ecological interactions between starlings and other cavity-nesting introduced bird species - such as the introduced common myna (*Acridotheres tristis*) (Pell and Tideman, 1997).

4.7. Invasiveness of a species

Understanding the invasiveness of a species (survival, fecundity and spread) and implicitly, the invasibility of recipient ecosystems with existing conspecifics, remains a major gap in our knowledge. This information will assist in predicting the penetration of a gene drive into an invasive population and its potential to spread (Backus and Gross, 2016). Although reintroduction biology research has advanced with respect to the recovery of native species (Armstrong et al., 2015), we know little about the invasiveness of the exemplar pest animals in this study within an Australian context. The survival of these species will be inherently linked to the invasibility of the receiving environment (Hui et al., 2016). Additionally, understanding gene flow, together with ecological changes, when the population of an invasive species is temporarily forced above natural levels is also important (Berry et al., 1991; David et al., 2013), especially in the context of introducing transgenic animals carrying a gene drive in an attempt to effect control. Implicitly, introduced species that have become invasive are anticipated to persist, breed and spread. However, the low scores for the exemplar species reflect a lack of information to support this assumption. Closing this gap would best be achieved by experimental releases of invasive species into areas where there are conspecifics to learn about the persistence, fecundity and potentially the additional ecological stress (e.g. lack of food, shelter) that comes from supplementing introduced individuals into an area.

4.8. Fertility control

A large body of work exists on the outcomes of experiments that simulate fertility control in mice (Chambers et al., 1999a, 1999b; Singleton et al., 2002), rabbits (Twigg et al., 2000; Williams et al., 2007) and foxes (Caughley et al., 1992; Twigg et al., 2000). Reduced fertility in these species has been demonstrated experimentally but density-dependent compensatory mechanisms – reproductive compensation by fertile females leading to increased progeny, and longer-lived progeny – were

also demonstrated (Williams et al., 2007). Information is currently lacking about breeding behaviour and reproductive performance of feral cats, cane toads, black rats and starlings that have been exposed to a level of sterilisation. Density-dependent models of transgenic mice using transgenic constructs that bias sex ratios predict that eradication efficiency varies based upon the fitness of the transgenic mice (Backus and Gross, 2016). It would also be useful to combine trials that simulate fertility control with male-bias in a population of a species of interest to understand the survival rates, reproductive rates and population dynamics for gene drive sex biasing research. Questions also worth pursuing relate to whether there are density-dependent implications to reproductive effort for a population with a higher number of males to females particularly for social species such as mice, rabbits and foxes.

5. Conclusions

The application of genome editing methods to pest management offers a promising new opportunity for the control of invasive species. Using RNA guided gene drives, based on CRISPR/Cas9, to control invasive species has potential to address the dilemma of broad-scale invasive species management programs across Australia, and elsewhere. Which invasive species should be considered a priority in each context will in part depend upon the information currently available and closing the gaps on the relevant information required.

Wild house mice and European rabbits may appear to be a logical first choice to develop and trial an RNA guided gene drive control strategy. Both have relatively short generation times, are easy to maintain under experimental conditions, and there is an extensive body of literature about their mating systems, ecology, and molecular biology. However, we found limited longitudinal (including gene flow) information available about the survivorship and reproductive potential of wild house mice (Berry et al., 1991) or wild rabbits that have been introduced into populations of conspecifics. Seasonality and a social reproductive structure, together with the presence of existing biological controls in the environment for rabbits, are the critical elements of mouse and rabbit life history that influence population levels in Australia (Tables A1 and A5). The gap is understanding the competitive reproductive performance of the targeted population (carrying a gene drive) upon introduction into a wild population of mice or rabbits.

The knowledge gaps within the context of gene drive development for the red fox are also relatively low. Although gene flow information within and between social groups is currently minimal, a largely monogamous mating system suggests gene drives will take longer to spread in this species.

Feral cats could be an attractive choice for gene drive research because of their serious direct impacts on native fauna. The knowledge gaps related to feral cats are moderate compared to the other invasive species assessed. Feral cat mating systems under free-ranging situations, gene flow between interacting feral, stray and domestic cats, their reproductive behaviours and interactions with conspecifics are areas that require further investigation. Although low fecundity and the development of transgenesis and animal husbandry will take much time, the severity of impact on native species means that this is not an impediment to pursuing gene drive population suppression in this invasive species.

Similarly, cane toads are an invasive species where community support may be easily gained for their control or local eradication; however, while high fecundity is an advantage for early molecular work on this species, there is no information to interpret how sex ratios are regulated in this species. Furthermore, high fecundity is offset by a long generation interval in this species. In modelling terms this will have a significant impact since gene drives spread from generation to generation not within a generation (like a viral vector or other external biological control agent). For black rats and starlings, the knowledge gaps are surprisingly large within the Australian environmental context. A lack of knowledge about the ecology and ecological role of black rats in Australia has been acknowledged elsewhere (Banks and Hughes, 2012), and is particularly lacking with respect to the factors that regulate their populations, home range and spatial overlap between individuals, and density-dependent reproduction. Advancing spatial models using empirical life history and gene flow data for these species would also enable the testing of hypotheses related to population regulation (including density-dependent reproductive rates) and the consequential effects on gene drives.

Interspecific breeding is a concern in the context of gene drive control as there is the possibility that hybridisation might produce negative ecological consequences through impacts on non-target species (Rhymer et al., 1994;). Except for black rats that may co-occur with several native *Rattus* species across Australia, none of our exemplar species coexist with native Australian species of the same genus, and there is no evidence to suggest that interspecific mating can occur. Intraspecific breeding between wild rabbits and domesticated rabbits, or feral cats and house cats, may occur over time, although the risks and consequence can be effectively managed through responsible pet ownership.

New integrated management tools for controlling invasive species are urgently needed. Acknowledging and assessing the risks of gene drive technologies is critical to the planning and communication process (National Academies of Sciences, Engineering, and Medicine, 2016). The biological and social risks and barriers can only be properly contextualised and mitigation designs evaluated with specific baseline information on the biology and ecology of target species (Dearden et al., 2017). There are many other invasive species across Australia – and globally in countries such as New Zealand, USA and Europe, for which the framework reported here could be applied. An explicit risk assessment process, and community acceptance of the approach, are areas that require further discussion and development to address the potential concerns associated with this biotechnology. Our systematic evaluation process and outcomes discussed here provide a solid basis on which to plan, contextualise and develop this innovative and potentially useful technology toward eventual deployment for the population control of invasive species in a field situation. While there is a pervasive need to engage the community with

the social licence to undertake gene drive research, calls to investigate new approaches for sustainable and economic landscape-wide alternatives to current invasive species population control or eradication need to also progress while being cognisant of the ecological risks.

Acknowledgements

We thank Peter Brown (CSIRO) for reviewing a draft of this work, and Lee Rollins, and John Woinarski for useful advice on current invasive species research. The authors declare no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.gecco.2017.e00363>.

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