Towards a genetic approach to invasive rodent eradications: assessing reproductive competitiveness between wild and laboratory mice

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Abstract House mice are significant invasive pests, particularly on islands without native mammalian predators. As part of a multi-institutional project aimed at suppressing invasive mouse populations on islands, we aim to create heavily male-biased sex ratios with the goal of causing the populations to crash. Effective implementation of this approach will depend on engineered F1 wild-lab males being effective secondary invaders that can mate successfully. As a first step in assessing this possibility, we are characterising genetic and behavioural differences between *Mus musculus* strains in terms of mating and fecundity using wild house mice derived from an invasive population on the Farallon Islands (MmF), a laboratory strain C57BL/6/129 (t^{w2}), and F1 wild-lab offspring. Mice with the 't allele' (t^{w2}) have a naturally occurring gene drive system. To assess fertility in F1 wild-lab crosses, t^{w2} males were paired with wild-derived females from the Farallon Islands (MmF). Results of these matings indicate litter sizes are comparable but that weaned pup and adult wild-lab mice are heavier in mass. Next, we initiated tests of male competitiveness using larger (3 m^2) enclosures with enrichment. We introduced both an MmF and a t^{w2}-bearing male to two MmF females to assess mating outcomes. Preliminary results of these experiments show none of the offspring carried the t-allele. However, performing the same experiment with F1 wild-lab males instead of a full lab background resulted in 70% of offspring carrying the t^{w2} allele. This indicates that F1 wild-lab males may be able to successfully compete and secondarily invade. It will be important in subsequent experiments to determine what characteristics contribute to secondary invasion success. More generally, a better understanding of characteristics contributing to overall success in increasingly complex and naturalistic environments will be critical in determining the potential of a gene drive-based eradication approach for invasive mice on islands.

Keywords: competition, gene drive, invasive rodents, reproductive fitness, secondary invasion

INTRODUCTION

Invasive rodents are a key biodiversity threat for the majority of the world's islands and eradication campaigns are often employed to prevent loss of island endemics (Howald, et al., 2007; Campbell, et al., 2015). These eradications employ rodenticides and have been successful in eliminating invasive rodents from over 400 islands (DIISE, 2017). Rodenticides, however, have a higher failure rate with mice (*Mus musculus*), as opposed to rats (*Rattus* spp.) (MacKay, et al., 2007) and their use on inhabited islands presents severe logistical challenges. Additionally, rodenticides are not species-specific and present animal welfare concerns (Campbell, et al., 2015). These challenges have created a compelling need for alternative approaches to rodent eradication.

One potentially promising approach to eliminating invasive mice from islands would be to bias offspring sex ratios by genetically engineering mice that produce only one sex of offspring. Pairing this approach with a genetic drive mechanism to spread this trait in an invasive mouse population would be critical. Key first steps are to understand the processes of reproductive competitiveness and the capability of an introduced mouse to introgress into established island populations, a process we are terming 'secondary invasion'. The phenomenon of secondary invasions and multiple introductions has been documented in invasive brown anole (*Anolis sagrei*) populations with evidence that secondary invasions may be frequent and can add genetic variation to existing invasive populations (Kolbe, et al., 2004). This secondary invader phenomenon in house mice, however, is less well understood and genetic evidence suggests variation in how this occurs across islands. Some studies suggest that secondary invaders may be frequent (Berry, et al., 1991; Bonhomme & Searle, 2012) while others suggest instead only single primary invasions (Hardouin, et al., 2010; Gabriel, et al., 2015). For rodent eradications these secondary invaders would be carrying the gene drive and spread of this construct through the population would be necessary for this approach to be effective.

The development of the CRISPR/cas9 genome editing technology has recently revolutionised genetic engineering capabilities (Barrangou & Doudna, 2016). This has increased interest in genetic pest management approaches first conceptualised by Burt (2003) and built upon by other authors more recently (Sinkins & Gould, 2006; Esvelt, et al., 2014). Many of these approaches centre on gene drives, systems in which a genetic construct producing a desired phenotype (e.g., sex ratio manipulation, sterility) is preferentially inherited by offspring. These are considered 'selfish' genetic elements because the majority of offspring will inherit the genetic construct and it therefore could spread quickly through a population (Lyttle, 1991). In mice, a naturally occurring gene drive is found on chromosome 17 and is termed the t-allele (Silver & Buck, 1993). The t-allele bearing sperm impact the motility of non-t bearing sperm and this leads to an inheritance rate of greater than 90% for the t-allele (Bauer, et al., 2005; Baker, 2008). Homozygosity of the t-allele (t/t) is typically lethal, but this is not true of the variant form termed the t^{w2} allele, although homozygosity does cause sterility (Levene & Dunn, 1961).

A gene drive-based approach to eradication could use either a naturally occurring drive or a synthetic drive based on CRISPR/Cas9 and functional drives with this technique have now been demonstrated in mosquitoes, flies, and yeast (Harris, et al., 2012; DiCarlo, et al., 2015; Gantz & Bier, 2015); see also early contributions by Craig, et al. (1960) and Hamilton (1967). Theoretically, by biasing offspring sex ratios heavily towards males, reproduction could be impaired and populations reduced. One way this could be done would be to use the *Sry* gene. The *Sry* gene is the key male determining factor in mammals and is sufficient to start the cascade of events leading to male development (Hacker, et al., 1995). Placing the *Sry* into an autosome induces development that is phenotypically male in mice that are genotypically XX (Koopman, et al., 1991). Inserting *Sry* into a naturally-occurring gene drive such as the t-allele or a synthetic drive based on

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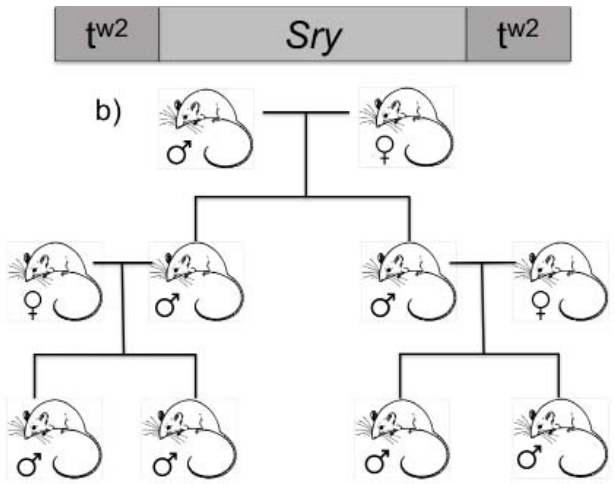

Fig.1 Depiction of the *Sry* gene inserted into the t^{w2} gene drive accompanied by a depiction of how the population would bias to be all male.

CRISPR/Cas9 should create the potential for reduction of an invasive mouse population by reducing and ultimately potentially eliminating production of fertile females (Fig. 1; Backus & Gross, 2016; Piaggio, et al., 2017; Prowse, et al., 2017). A synthetic gene drive using CRISPR/Cas9 could theoretically be employed in a similar way to ensure all offspring inherit a feminising gene.

Regardless of the genetic mechanism employed, the reproductive competitiveness and relative fitness of gene drive carriers are likely to be important in determining the success of any genetic approach to reducing invasive mouse populations. Assessing reproductive competitiveness is the focus of this study. Since mice introduced with a gene drive mechanism would essentially be secondary invaders into an established invasive mouse population, it is important to better understand processes affecting introgression into established demes. Mice are social animals and dominant males will often hold and defend a territory (i.e. deme) that provides reproductive access to reproductive females while subordinate males do not (Bonhomme & Searle, 2012). How incoming mice are able to successfully integrate into island demes is not clear. If a gene drive approach was used, then the incoming males would need to compete with the resident island males for females. Competition and aggression tend to occur between male mice when there are limited territories (Gray & Hurst, 1998). Mouse populations living non-commensally on islands can instead exhibit an 'island syndrome' where they show important differences with commensal populations. These can include increases in body mass and, importantly in the context of this study, lower levels of aggression (Adler & Levins, 1994; Gray & Hurst, 1998; Cuthbert, et al., 2016). In the 1980s, a study was conducted by capturing house mice on the Orkney island of Eday (commensal) and releasing them onto the Isle of May, which was uninhabited by humans but had an established population of non-commensal wild house mice (Berry, et al., 1991). This study followed the spread of genetic markers unique to Eday and found that these alleles moved quickly through the Isle of May population (Berry, et al., 1991; Jones, et al., 1995). Differences in aggression may relate to whether the mice are living commensally or not, with evidence indicating that commensalism and perhaps increased density favours more aggressive individuals (Berry, et al., 1991; Gray & Hurst, 1998). Overall, the limited studies to date have strongly suggested that island mice may not be as competitive as their mainland/commensal counterparts (Mackintosh, 1981; Berry, et al., 1991; Gray & Hurst, 1998).

Secondary invader success may also depend on female mate choice (Jones, et al., 1995). In terms of female mate choice, there is evidence that females prefer the scent of foreign males and are more likely to mate with unrelated males (Roberts & Gosling, 2003; Frynta, et al., 2010). Importantly, however, there is also evidence of female choice favouring non-t haplotype carrier males or males carrying a different t-haplotype variant (Lenington et al., 1994; Manser, et al., 2015; Sutter & Lindholm, 2016). The relative fitness of gene drive carriers will be a critical determinant of effectiveness for this approach. Fitness costs have been documented with other forms of the t-allele (Carroll, et al., 2004; Lindholm, et al., 2013), but have not been examined for the t^{w2} variant to our knowledge. Information about the t-allele presence on islands and modelling of population dynamics would help us further understand the transmission of the Sry/t^{w2}gene drive in island mouse populations (Backus & Gross, 2016).

Central questions

A critical aspect of exploring gene drive eradication techniques for island rodents is that the gene drive originates in a mouse strain with a standard laboratory background that is amenable to manipulation. Laboratory mice, however, have been inbred and housed in non-hierarchical social conditions for generations (Morse, 2007; Fawcett, 2012) and they have also undergone both deliberate and inadvertent selection under these captive conditions (Fawcett, 2012). It is encouraging to note, however, that wild-type behaviour can be restored quickly by backcrossing with wild-derived mice to create wild-lab crosses (Chalfin, et al., 2014). The central goals of this study are to one i) confirm that a gene drive mechanism can be bred into a wild background and ii) assess whether key reproductive measures such as litter size, pup weight, and adult weight are impacted in F1 and F2 wild-lab mice. We also present preliminary findings regarding the success of laboratory and F1 wild-lab males in competitive mating situations.

MATERIALS AND METHODS

Strains of mice

These studies employed several different strains of mice. A primary laboratory strain is C57BL/6J referred to as (B6) mice. B6 mice are the most common strain of lab mice and are easily manipulated genetically (Silver, 1995). Compared to other laboratory strains B6 mice are considered more defensive and aggressive in response to perceived threats (Blanchard, et al., 2009). A second strain was donated from the Threadgill lab at Texas A&M University. These mice are of a mixed C57BL/6J and a 129S1/SvlmJ (B6;129) background (hereafter referred to as "lab" strain) and carry the t^{w2} variant of the t-allele. The t^{w2} variant stems from a wild background but was brought into laboratory stocks in 1946 (Dunn & Morgan, 1953). These mice are not transgenic (no *Sry* inserted) and so heterozygotes produced are either male or female. The t^{w2} allele is inherited by 95% of offspring in matings with a t^{w2}/+ sire (Kanavy & Serr, 2017). To maintain t^{w2} mice, B6 females are mated to males heterozygous for the t^{w2} allele (t/+). The wild-derived mice (MmF) we use are derived from wild progenitors captured on Southeast Farallon Island, which is part of the Farallon National Wildlife Refuge, located about 30 miles off the coast of California near San Francisco (Farallon, 2013). Invasive mice are the only terrestrial mammals on the island currently (Schoenherr, et al., 1999; Farallon, 2013). These mice show annual cyclic population variation with peak densities in late summer and early fall. MmF mice do not carry the t allele (Threadgill, pers. comm. 2013).

Some of the highest mouse densities ever recorded in non-commensal habitats are seen on Southeast Farallon Island at over 1300/ha (490/acre) (Farallon, 2013; Newser, 2013). Their diet consists primarily of invertebrates (Jones, et al., 2006). The Farallons mice pose direct threats to an endemic invertebrate and indirect threats to native seabirds. The USFWS plans for a future mouse eradication with rodenticide (Farallon, 2013). We established a colony of wild-derived Farallons mice (MmF) at NCSU in 2013 and they are now 8th generation derived from the wild. These Farallon mice serve as the 'island mouse' model being used to form demes for testing the ability of secondary invaders to establish and mate successfully.

All experiments were conducted under an approved Institutional Animal Care and Use Committee protocol at North Carolina State University between 2015–2017. Mice were maintained in a temperature-controlled greenhouse with natural lighting and conditions suitable for reproduction year round. Animals were fed *ad libitum* with 5058 LabDiet® and daily health and welfare checks were performed. To test if mating between wild-derived MmF females and laboratory males occurred pairs of lab males with wild-derived MmF females were created and housed in 29 cm wide × 40 cm long × 19 cm high standard laboratory cages. Each cage contained aspen bedding, natural cotton, a 15 cm PVC tube and black oil sunflower seeds for enrichment. Mice were housed in this manner with weekly cage changes. To minimise disturbance, mice were transferred over to a clean cage using a 15 cm PVC pipe whenever possible. Pups were weaned at the mouse standard of 21 days +/- 3 days (Silver, 1995) and the litter size, sex and weight of the pups in grams were recorded. In addition, an ear punch or tail snip was taken for genotyping. Pups were then weighed as adults and their weight in grams was collected for nulliparous individuals between the ages of 70–140 days.

Tests of male competition were conducted in semi-natural enclosures. The size of these 'arenas' is 3 m^2, closely approximating the size of those used by Slade, et al. (2014). To allow for formation of hierarchies and nesting, we added enrichment and complexity in the form of sand, bricks, plastic blocks ('Legos') supporting multilevel clear Plexiglass structures, galvanized wire mesh (1.25 × 1.25 cm mesh size), cardboard boxes and cardboard egg cartons, and PVC pipes for environmental complexity. For trials, all mice were placed into the arena at the same time. Males were either weight matched to within 1 g (~5% of body weight) or age matched within 8–10 weeks. All mice used in the arenas were nulliparous and sexually mature. Coloured ear tags as well as Clairol 'Just For Men' Black Hair dye® was used to identify males. Trials included combinations of MmF and t^{w2} males as well as MmF and F1 wild-lab males. At the start of each trial, both males and two non-related MmF females were placed into the arena and filmed for one hour. During this hour, we counted the number of bouts, chases and attempts to copulate, or time in proximity with females, as a means of assessing dominance. Animal welfare checks and monitoring for pups were performed daily. Any pups born in the enclosures were weaned at the standard of 21 days and a tissue sample was collected for genotyping.

To confirm the presence of the t^{w2} haplotype, we used a modified protocol where we amplified a portion the Hba-ps4 (alpha-globin pseudogene-4) locus (Schimenti & Hammer, 1990). The procedure uses a 'dirty' DNA extraction developed by one of our collaborators at Texas A&M University (Kanavy, pers. comm. 2016). Tissue is collected and either a 2–3 mm tail snip or a 2 mm ear punch is used. The 'dirty' DNA extraction buffer contains (50 µl 5 M NaOH, 4 µl 0.5 M EDTA, and 10 ml sterile water). 100µl of extraction buffer is then added to the tissue sample and incubated at 95°C for 20 minutes. After vortexing and cooling 5 µl of 1 M HEPES is added. The sample is then centrifuged at 6,000 g for five minutes and 40 µl of DNA is extracted from the top. DNA electrophoresis of PCR products shows a distinct band at 198 bp for wildtype mice (+/+) while t^{w2} homozygotes (t/t) display a band at 214 bp and heterozygotes (t/+) show the presence of both bands.

Statistical analyses were conducted using JMP® Pro 12.2.0 (SAS) where 1-way ANOVAS were used for adult weights and litter sizes. A mixed model ANOVA with the fixed effect of litter size was used to separate litter size from pup weight to compare pup weights. Next, post-hoc analyses including orthogonal contrasts and Tukey's HSD tests were used to identify group differences. Litter sizes and weights are presented as mean ± SEM.

RESULTS

Adult weights were taken for males and females. Sample sizes for males were as follows: B6 (33), t^{w2} (24), MmF (53), F1 (21), and F2 (22). For females sample sizes were: B6 (19), t^{w2} (25), MmF (44) and F1, (23). The average day of age that adult male weights were measured at was the following: B6=80.43± 21.95; t^{w2} =90.43±27.65; MmF 92.63±34.90; F1 93.03±19.46; and F2 89.48±28.27. Similarly, for females the average day of age that the adult weight was taken was: B6 91.24±28.99; t^{w2} 88.66±24.09; MmF 89.20±36.14; and F1 82.25±38.15. Adult weights varied by strain and sex, $F_{8,257}$=28.35, p<0.0001. In addition, t^{w2}carrying males (t^{w2}, F1, F2) were larger than MmF males, F=58.00, p<0.0001. Similarly, t^{w2} carrying females (t^{w2} and F1) were larger than MmF females, F=7.75, p=0.0058 (Fig. 2). Due to space restrictions for husbandry, not enough F2 adult females had been reared to allow calculation of a meaningful average for this group.

While litter size varied across strains $F_{5,141}$=4.59, p<0.0007, MmF, F1 and F2 wild-lab mice had litter sizes that were comparable (Fig. 3). Sample sizes for litter size were as follows: B6 (27); t^{w2} (20); MmF (45); MmF/B6 (19); F1 (21); and F2 (20). There were no differences detected in the sex ratios for pups born, nor in the time of gestation (data not shown).

Weaning weight was measured with a mixed model ANOVA with litter size being a fixed effect. The samples are as follows: B6 (18); t^{w2} (14); MmF (44); MmF/B6 (20);

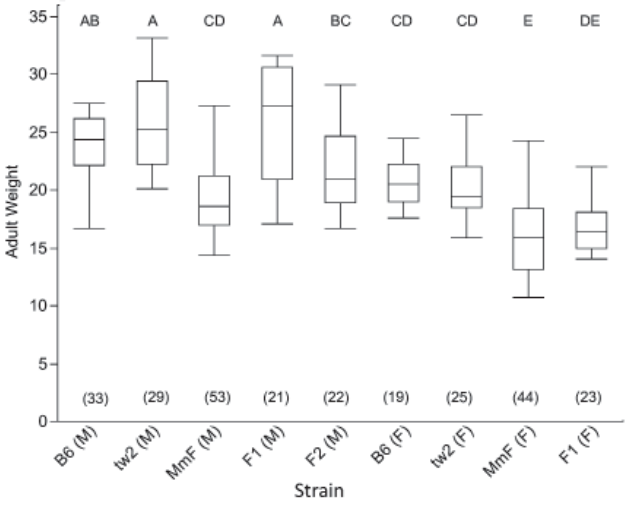

Fig. 2 Adult weight by strain and sex. 1-way ANOVA, $F_{8,257}$=28.35, p=0.0001. Tukey's HSD reveals significant differences in weights indicated by letters. Sample sizes are indicated in parentheses.

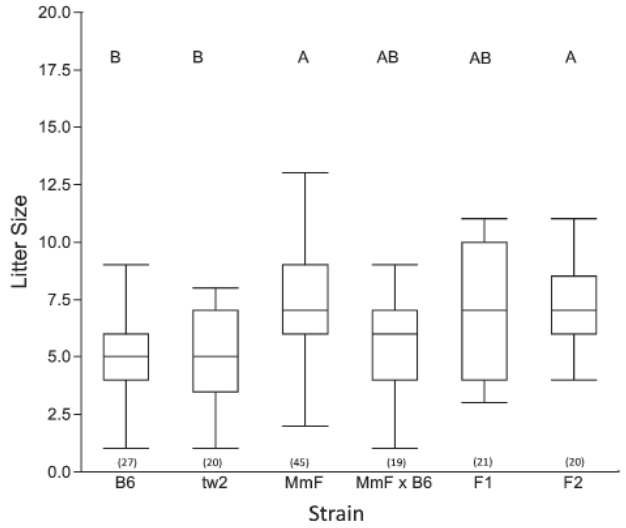

Fig. 3 Litter size by strain 1-way ANOVA, $F_{5,141}=4.59$, $p=0.0007$ indicates significant differences in litter size across strains. Tukey's HSD reveals significant differences in weights indicated by letters. Sample sizes are indicated in parentheses.

F1 (13); and F2 (20). Pup weaning weight was significantly different across strains ($F_{133,383}=13.922$, $p=0.0001$) and the highest weaning weights were found in F1 wild-lab F2 and F1s respectively (Fig. 4). Highest mean weights at weaning were 10.46± 0.40 g (F1) and 9.82± 0.33 g (F2).

In the arenas, preliminary trials of male competition between t^{w2} males (laboratory strain) and MmF males revealed no t^{w2} transmission based on genotyping (three trials with 35 pups total). The t^{w2} male initially appeared behaviourally dominant. He pursued females and chased the MmF male away, but on subsequent days was subordinate and tended to stay on top of the feeder out of view of the MmF male. Preliminary trials with MmF males and F1 wild-lab males (eight trials, 47 pups) revealed strongly contrasting results and a 70% transmission rate of the t^{w2} allele. Here, five of the eight litters did carry the t^{w2} with 31 of 33 pups from these litters confirmed. The F1 wild-lab males appeared to be behaviourally dominant throughout the trial in the same five trials where t^{w2} pups were produced.

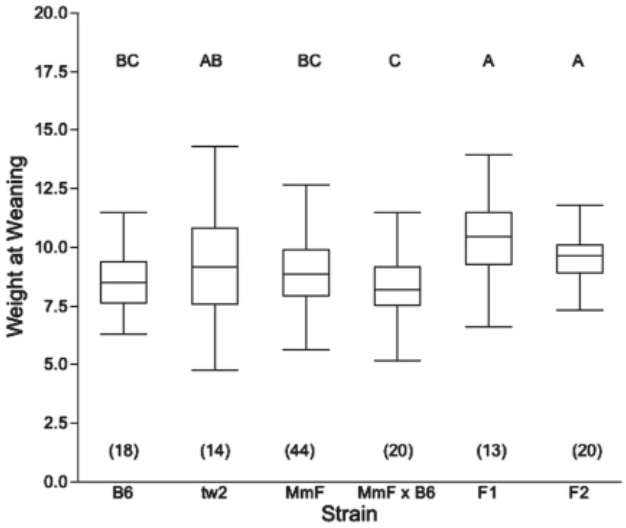

Fig. 4 Weaning weight with fixed effect of litter size Mixed Model, strain $F_{5,119}=4.98$, $p=0.0004$., litter size $F_{1,117}=12.46$, $p=0.0006$. Tukey's HSD reveal significant differences in pup weights across strains, which is indicated by letters. Sample sizes are indicated in parentheses.

Dominance was again based on initiation of chasing or fighting with the MmF male and by time spent pursuing or mating with females. When subordination did occur, the subordinate males appeared to place themselves so as not to be visible to the dominant individual. Behavioural results are ongoing and were beyond the scope of this manuscript.

DISCUSSION

Relative fitness of gene drive carriers is likely to be critical in determining the success of this approach (Burt, 2003; Manser, et al., 2015; Backus & Gross, 2016). Carriers of gene drive constructs would need to be successful in reproduction and reproductive competition if a genetic approach to invasive rodent eradication is to be effective. This work establishes some key initial conditions for this success. First, lab mice and wild mice can breed and produce viable litters. Second, while litters of the common lab background t^{w2} mice were smaller than those of wild-derived mice under the more naturalistic conditions used in this study, the F1 wild-lab litters were of comparable size to those having two wild-derived parents. Preliminary results also suggest F1 wild-lab males may have strong potential for reproductive success, a likely prerequisite for initial introgression of gene drive constructs into an island population.

This work established that wild-derived Farallon females will mate with laboratory males in standard cages and at similar frequencies to those seen in matings with wild-derived males (M. Serr, unpublished data). This was an initial but critical step in assessing reproductive output across strains and in F1 wild-lab mice. Furthermore, results indicate that both F1 wild-lab and F2 wild-lab backcrossed mice have litter sizes that are not different statistically than those of Farallon mice. This is important in terms of fitness and exploring the effectiveness of using the Sry/t^{w2} haplotype technique. It is also important to note that the reverse holds true, as wild-derived MmF males will mate with B6 and t^{w2} females in standard laboratory cages although sample sizes are not adequate for statistically meaningful comparisons. Results for pup weights indicate F1 and F2 wild-lab pups have the greatest weight at weaning and that this trend continues for adult males. Body size affects male competitiveness in mice (Cunningham, et al., 2013; Ruff, et al., 2017) with evidence suggesting that in semi-natural enclosures male mice of intermediate weight have the highest fitness (Ruff, et al., 2017). Matching mice based on body size for our experiments helps rule out this confounding factor, but for a potential gene drive release it could be beneficial for the drive-bearing mice released to weigh more than their wild counterparts.

Preliminary results from experiments in our larger arenas examining competition suggested a surprising pattern. Arena trials between MmF and t^{w2} males suggest the wild-derived MmF males are dominant to pure laboratory strain males, preventing transmission of the t^{w2} allele. Interestingly, however, weight-matched F1 wild-lab males carrying the t^{w2} allele appear more competitive and behaviourally dominant to MmF males. Consistent with this observation, we find a 70% transmission rate of the t^{w2} allele in arena trials analysed thus far. In addition, of the three trials where the F1 wild-lab male was not dominant MmF litter sizes were small with two of the three litters only having two pups each. This suggests that F1 wild-lab males are strong competitors and that females will mate with F1 wild-lab males even when both male types are present. It will be important to conduct further arena trials to assess this competitiveness with greater sample sizes and also assess the competitiveness of F2 wild-lab males. Other reproductive comparisons we are conducting include measuring testes weights. Testes weight is correlated to

total sperm count in mice (Le Roy, et al., 2001). Testes weight can also predict dominance and mating success, as mice with higher testicular weight are more likely to initiate mating with females and attack behaviour towards conspecific males (McKinney & Desjardin, 1973). Finally, nesting behaviour and the temperature of nests will be important to examine across wild-derived, laboratory and F1 wild-lab mice as anecdotal observations suggest poor nest construction by laboratory mice. This could be important too because in cooler environments studies have indicated that nest building behaviour, thermoregulation, and fitness are correlated (Bult & Lynch, 1997).

Our results suggest that F1 wild-lab males could be efficient secondary invaders. This would be generally consistent with other studies from island populations (Jones, et al., 1995; Bonhomme & Searle, 2012). However, the situation may be different for females. Introduction of mice from a commensal population on the Isle of Eday to the Isle of May did not lead to the spread of mitochondrial DNA markers, which are maternally inherited. These results were in contrast to those for a Y-chromosome marker and suggested females were unable to secondarily establish while males did (Jones, et al., 1995). Studies from other islands have corroborated these results in suggesting no integrations of new maternal haplotypes from later-arriving females (Searle, et al., 2009; Gabriel, et al., 2010; Jones & Searle, 2015). This apparent male-female asymmetry in secondary establishment ability, however, has not been experimentally tested. One approach to addressing this apparent asymmetry is having records of detailed behaviour in more naturalistic arena settings. We have designed and implemented a Radio Frequency Identification (RFID) system for tracking mouse movements. RFID tracking allows collection of detailed behavioural records and works well with wild house mice (Weissbrod, et al., 2013; Auclair, et al., 2014). Behavioural measures include time spent at nest boxes, running wheels and food. With this information we can assess the number of visits, the timing of visits, the number of interactions and time in social contact with one another (König, et al., 2015; Lopes, et al., 2016).

A second approach is to test the ability of different strains to establish dominance in a standard test termed resident-intruder paradigm. A previous study used this approach to compare competitive behaviour in house mice from the Isle of Eday and the mainland, finding the island mice were significantly less aggressive (Gray & Hurst, 1998). Expanding trials to increasingly complex naturalistic experimental arenas should give insight into the relative abilities of male and female mainland mice to secondarily invade and therefore genetically introgress into an island population.

Other factors that could influence the potential success of an eradication effort include mate-choice and tolerance of island conditions. Mate-choice factors known for mice include odorant cues such as urinary proteins and ultrasonic vocalisations (Hurst & Beynon, 2004; Blanchard, et al., 2009; Musolf, et al., 2010). Island conditions and climate, in particular, could be important influences on the success of introduced mice (Berry, 1992). The island syndrome for rodents predicts increased body mass and decreased aggression (Adler & Levins, 1994; Gray & Hurst, 1998; Cuthbert, et al., 2016). In addition, the island syndrome in rodents is often associated with high population densities, increased reproductive output, and increased survival rates on islands (Adler & Levins, 1994). Mice are able to adapt to new conditions and islands (Anderson, 1978; Bronson & Pryor, 1983) and this adaptation could be critical for fitness, although any construct would presumably be introgressed into an island genetic background relatively quickly as it spread. The population genetic structure of the mice already present on an island would be critical for a synthetic gene drive, but other factors including the rate of inbreeding, ratio of reproductive males to females, and age structure of the mouse population(s) might also prove important. These are also likely to impact spread of either a synthetic or natural drive like the t-haplotype considered here. In regions with seasonality and temperature variations, mouse populations often undergo a 'boom and bust' cycle, as seen in the Farallon Islands, where the populations can erupt only to die off with changes in temperature. The timing of release of secondary invaders will likely be important in these situations (Singleton, et al., 2005; Farallon, 2013; Backus & Gross, 2016). Both natural and sexual selection could influence the number of drive carrier mice that would be required for eradication success. A study by Backus & Gross (2016) modelling the Sry/t^{w2} gene drive found that the relative fitness of the mice carrying the gene drive determined whether multiple releases would be required. Similarly, Prowse, et al. (2017) modelled synthetic gene drives and found that a sex reversing drive would require multiple releases to achieve eradication success.

The concept of reducing invasive mouse populations through release of genetically-modified mice is still in the early stages of development. Many key issues will need to be addressed to determine whether this is a feasible approach. We have shown that an island-derived wild strain will mate with t^{w2}-carrying laboratory males and produce comparable litter sizes to those of wild–wild matings. Promisingly, we also see that pup-weaning weights are larger for F1 and F2 wild-lab mice and that F1 wild-lab males may be stronger competitors in semi-natural enclosures. A key future step will be to scale up trials in arena size and environmental complexity. Larger enclosures could be used with greater numbers of mice to test whether a gene drive can spread under controlled and biosecure, but naturalistic conditions. Finally, beyond the technical issues discussed above, social license for any environmental releases would be crucial (NASEM, 2016). As gene drives are a new technology still in development, input from the relevant publics and regulatory authorities will be very important moving forward and this input is also likely to lead to additional interesting and important questions that developers will need to address.

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