Genetically Engineering Wild Mice to Combat Lyme Disease: An Ecological Perspective

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Genetic engineering of wild populations has been proposed for reducing human diseases by altering pathogens' hosts. For example, CRISPR-based genome editing may be used to create white-footed mice (Peromyscus leucopus) that are resistant to the Lyme disease spirochete vectored by blacklegged ticks (Ixodes scapularis). Toward this goal, academic researchers are developing Lyme-resistant and tick-resistant white-footed mice, which are a primary pathogen reservoir for Lyme disease in the United States. If field trials on small, experimental islands are successful, the project would scale up to the larger islands of Nantucket and Martha's Vineyard, Massachusetts, and possibly to the mainland, most likely with a local gene drive to speed the traits' proliferation, pending approvals from relevant constituents. Despite considerable publicity, this project has yet to be evaluated by independent professional ecologists. In the present article, I discuss key ecological and evolutionary questions that should be considered before such genetically engineered mice are released into natural habitats.

Keywords: Borrelia burgdorferi, genome editing, Ixodes scapularis, Peromyscus leucopus, risk assessment

Rapid advances in genetic engineering (GE) have led to a host of proposed applications, including the use of both genome editing and gene drives to begin a new era of "ecological engineering" (Esvelt et al. 2014, Buchthal et al. 2019). Specifically, CRISPR technology is easier to use than previous transgenic methods and allows researchers to delete, alter, or insert desired genetic sequences into known target sites of an organism's genome (Doudna and Charpentier 2014). For example, CRISPR-based genome editing could be used to introduce a beneficial trait such as pathogen resistance into populations of a threatened species (Piaggio et al. 2017). To promote rapid fixation within populations, the new gene can be inserted along with a gene drive system that causes biased inheritance of genetic elements and thereby ensures very high rates of transmission of the inserted gene to subsequent generations (Esvelt et al. 2014). In sexually reproducing species with short generation times, gene drives can theoretically be used to push selectively beneficial, neutral, or even deleterious traits to fixation, including sterility genes for eradicating unwanted populations (Harvey-Samuel et al. 2017). These possibilities have prompted molecular biologists to extend the scope of their research to include wildlife management, epidemiology, and biological conservation (Piaggio et al. 2017).

In contrast to current transgenic crops or farm-raised transgenic fish, some future GE applications will focus on wild or feral species that will be able to reproduce and disperse freely in the environment. Indeed, the ability of introduced genes to spread throughout surrounding populations will be essential for achieving intended outcomes. For example, using older techniques, the once-common American chestnut (*Castanea americana*) has been genetically engineered to resist the chestnut blight fungus that nearly drove this species to extinction (Popkin 2018). If transgenic chestnut strains receive regulatory approval, their introduced resistance genes will be able to spread freely via pollen and seeds into unmanaged habitats (NASEM 2019). In a similar vein, as is described in the present article, GE Lyme-resistant white-footed mice (*Peromyscus leucopus*) are being developed to combat Lyme disease by proliferating freely on the islands of Nantucket and Martha's Vineyard, Massachusetts. This project is designed to be a first step toward larger scale introductions on the mainland and does not involve a gene drive (Buchthal et al. 2019).

To date, much of the scientific literature on expected risks of using genome editing and gene drives in wild species describes complications that could undermine success (i.e., the risk of failure), such as naturally occurring mutations that inactivate an introduced DNA sequence (e.g., Champer et al. 2018). The need for careful environmental risk assessment has been widely acknowledged (e.g., Webber et al. 2015, NASEM 2016, 2017, Harvey-Samuel et al. 2017), but

BioScience XX: 1–11.
doi:10.1093/biosci/biz080

Figure 1. The white-footed mouse, **Peromyscus leucopus.** *White-footed mice typically survive for less than 1 year; females have about two litters per year, with three or four pups per litter (Spielman et al. 1981, Wolff 1985, Vessey and Vessey 2007). Photograph: Michael Richardson.*

realistic case studies involving free-living GE vertebrates are scant, in part because this field is so new. Commendably, many peer-reviewed papers and public workshops have focused on ethical implications of genetically engineering wild species and the importance of communication with members of affected societies (e.g., Kofler et al. 2018, Brossard et al. 2019). Nonetheless, public engagement about potential benefits and risks is incomplete without considering existing ecological expertise and knowledge relevant to each proposed application (NASEM 2016). Furthermore, early involvement of independent ecologists could provide helpful guidance to molecular biologists who may not be aware of research gaps that could hinder success.

Here, I examine a case study involving the white-footed mouse (figure 1) in the context of broader questions regarding risk assessment for free-living GE vertebrates. As is widely acknowledged with previous types of transgenic organisms, each proposed use of genome editing in wild species should be assessed on a case-by-case basis to examine why, how, when, and where these techniques would be used (e.g., Snow et al. 2005, NASEM 2016). Below, I describe the ecological setting for this case study and the types of questions that should be evaluated before a GE mouse is approved for release into natural habitats. Although regulatory agencies in the United States and elsewhere have little experience with such nonagricultural applications to date, many questions about potential environmental risks of releasing GE vertebrates are similar to those described in a position paper by the Ecological Society of America for all genetically engineered organisms (table 1; Snow et al. 2005).

The Mice Against Ticks project

Kevin Esvelt, an assistant professor at the Massachusetts Institute of Technology (MIT), is a strong proponent for

Table 1. Possible environmental risks of releasing genetically engineered (GE) vertebrates into natural habitats (adapted from Snow et al. 2005), with examples of hypothetical, worst-case scenarios for the current case study involving GE Lyme-resistant white-footed mice.

Type of risk	Hypothetical scenario
Exacerbating effects of existing pests or pathogens.	***Scenario 1:*** *Competitive release of more harmful tick-borne pathogens that may currently be suppressed by the frequent presence of Lyme spirochetes in white-footed mice.* ***Scenario 2:*** *Increased unwanted contact between humans and white-footed mice during any massive, pulsed introductions of tens to hundreds of thousands of GE mice.*
Facilitating the introduction and establishment of new pests or pathogens.	*No proposed scenario.*
Loss of genetic diversity within species.	***Scenario 1:*** *Genetic bottlenecks that could occur during initial selection, lab-rearing procedures, and field releases of GE white-footed mice, perhaps leading to inbreeding depression, the loss of subspecies, or the loss of adaptation to local environments.*
Harm to other species, in some cases leading to a loss of species diversity.	***Scenario 1:*** *Fitness costs or benefits associated with a novel GE trait in white-footed mice, resulting in altered abundance or population fluctuations, with unwanted cascading effects on other species.* ***Scenario 2:*** *Altered foraging behavior of GE Lyme-resistant white-footed mice, such as preying on eggs of ground-nesting birds to a greater extent when white-footed mice are not infected by Lyme spirochetes (Ostfeld et al. 2018b).*
Other unwanted disruption of biotic communities, including disruption of ecosystem services.	*No proposed scenario.*
Noncompliance with legal or regulatory requirements, or with ethical standards for research and deployment of GE animals.	***Scenario 1:*** *Unintended dispersal and establishment of GE Lyme-resistant white-footed mice on the mainland or on other islands where regulatory approvals, environmental risk assessments, or public engagement are lacking. Long distance dispersal could occur via swimming or when white-footed mice become stowaways in boxes, gear, firewood, and other items that are transported by people (e.g., Scheppe 1965).*

Note: See the text for details. The environmental benefits of releasing GE vertebrates (e.g., efforts to preserve endangered species) are not considered in the present article, nor are cases that involve gene drive systems.

the responsible use of genome editing and gene drive technology in wild species (e.g., Esvelt et al. 2014, Esvelt and Gemmell 2017, Nobel et al. 2018). He and Sam Telford III, of Tufts University, identified Lyme disease as a target public health problem, and their Mice Against Ticks project has been underway since 2016 (Buchthal et al. 2019). This project involves engineering wild-caught white-footed mice to introduce two traits—resistance to the Lyme pathogen and, if feasible, resistance to feeding by the pathogen's tick vector.

In an effort to encourage transparency and public oversight, the Mice Against Ticks project involves public meetings, media attention, and local steering committees that can recommend terminating the project on their island (Buchthal et al. 2019). Also, several of the team's grant proposals and community presentations are available online at *www.responsivescience.org/mice-against-ticks*. As with any ambitious, long-term research project, the research plan is likely to be modified as the work progresses, pending initial outcomes and benchmarks. For the present article, I obtained feedback from Kevin Esvelt and his team to ensure that my summary of their ongoing research is up to date and accurate at this early stage of development.

Ecology of Lyme disease. Lyme disease is caused by a tick-borne spirochete bacterium, *Borrelia burgdorferi*, which is vectored by *Ixodes scapularis*, commonly known as the blacklegged tick (e.g., Lane et al. 1991). The blacklegged tick takes blood meals from many vertebrate species (Anderson 1988), some of which also serve as reservoir hosts that sustain spirochetes and transmit them back to new generations of ticks (figure 2, table 2). Ticks require a single blood meal during each stage of their 2-year life cycle, as larvae, nymphs, and adults (females only); larvae and nymphs feed primarily on small mammals, whereas female adults feed primarily on deer (figure 2; e.g., Anderson 1988, but see Huang et al. 2019). Most human infections are acquired from nymphs (Piesman et al. 1987, *www.cdc.gov*; see supplemental figure 1).

Infected ticks rarely transmit Lyme spirochetes to their offspring, which must feed on a reservoir host to become infected (e.g., Piesman et al. 1986). The white-footed mouse is often considered to be one of the most effective and abundant reservoir species for Lyme spirochetes in the northeastern United States (Piesman and Spielman 1979, Donahue et al. 1987, Lane et al. 1991, LoGiudice et al. 2003), but masked shrews (*Sorex cinereus*), short-tailed shrews (*Blarina brevicauda*), and chipmunks (*Tamias striatus*) also can function as reservoir species (table 2; Telford et al. 1990, Brisson et al. 2008, Ostfeld 2011). For example, in a New York forest, Brisson and colleagues (2008) estimated that shrews fed 55% of infected ticks, whereas white-footed mice fed only 25%. In this region, it appears that the majority

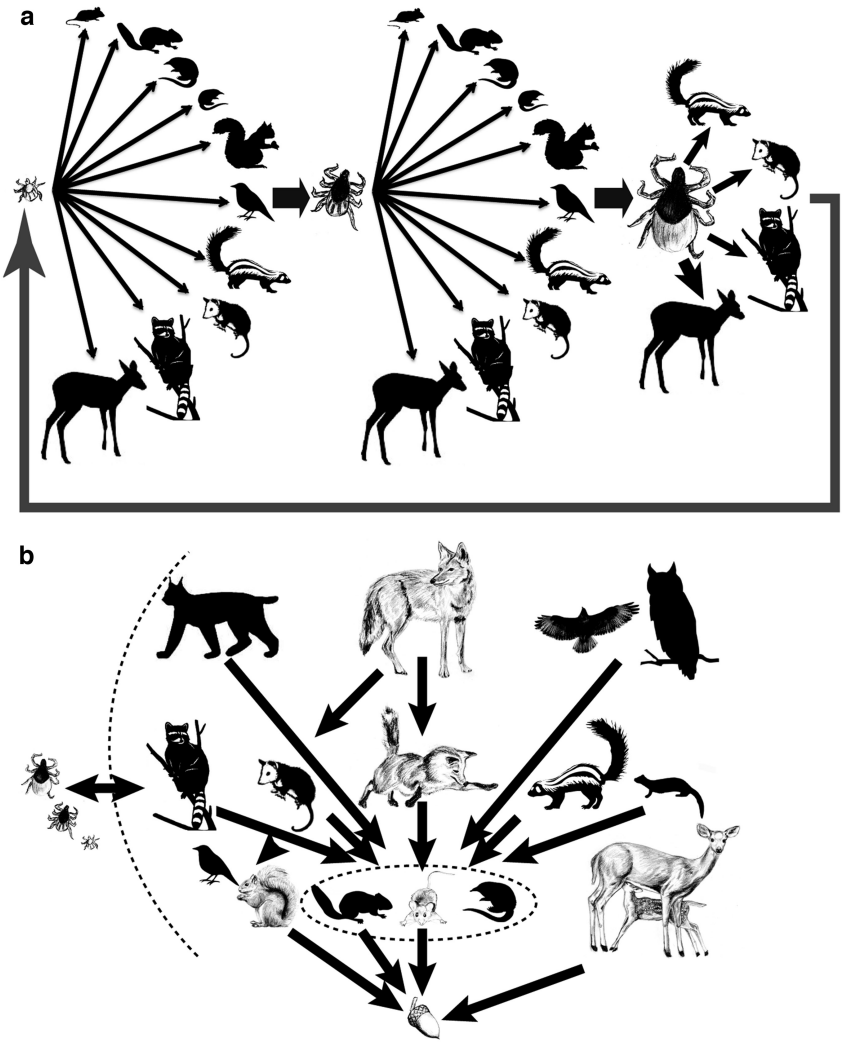

Figure 2. Schematic of ecological interactions that influence the prevalence of Lyme-infected ticks in the northeastern United States. (a) Blood meal hosts for blacklegged ticks at each stage of their 2-year life cycle (as nymph, larva, and adult). (b) Predators and competitors within the vertebrate host community, focusing on chipmunks, white-footed mice, and shrews, which serve as reservoir hosts for the Lyme spirochete (table 2). See the text for details and supplemental table 1 for the species found on the islands of Nantucket and Martha's Vineyard versus the mainland. Reprinted with permission from Levi and colleagues (2016).

of infected larvae and nymphs may feed on reservoir host species other than white-footed mice (LoGiudice et al. 2003, Ostfeld et al. 2018a). Nonetheless, large-scale field experiments in Connecticut and New York showed that the vaccinating white-footed mice against Lyme decreased the local frequency of Lyme-infected nymphs (Tsao et al. 2004, Richer et al. 2014). On coastal islands that lack populations of white-footed mice, Lyme transmission can occur because of other reservoir species such as the deer mouse, *Peromyscus maniculatus* (Isle au Haut, Maine; Rand et al. 1993); Norway rat, *Rattus norvegicus* (Monhegan, Maine; Smith et al. 1993); and meadow vole, *Microtus pennsylvanicus* (Patience Island, Rhode Island; Markowski et al. 1998).

On Nantucket and Martha's Vineyard, which lack many mainland mammal species (supplemental table 1), white-footed mice are the most common reservoir species captured in live traps (Spielman et al. 1981). Masked shrews, short-tailed shrews, and rats (*Rattus norvegicus*), are present on both islands, as are chipmunks on Martha's Vineyard (table 2, supplemental table 1). Shrews are more difficult to census accurately than white-footed mice, and standard live-trapping methods underestimate their abundance (Harder et al. 2014). In Ohio woodlands, Harder and colleagues (2014) found that shrews were as common as white-footed mice on the basis of data from pitfall traps in addition to live trapping. Shrew population densities on Nantucket and Martha's Vineyard have not been reported, so their relative importance in the Lyme disease cycle is not known.

Efforts to intervene and reduce human exposure to Lyme bacteria are complicated by a dynamic network of interacting species in this pathogen–vector–host community (figure 2; Eisen et al. 2017). Previous investigators have examined many factors that affect the prevalence of spirochete-infected ticks, including small mammal populations that are highly variable in time and space, masting oaks (*Quercus* spp.) and other nut-producing trees that sustain rodents and white-tailed deer (*Odocoileus virginianus*), effects of predators such as red fox (*Vulpes vulpes*), coyote (*Canus latrans*), and bobcat (*Lynx rufus*) on small mammal populations, and local densities of deer, which are a major host for adult ticks but do not function as reservoir species (e.g., Ostfeld et al. 2006, Levi et al. 2012, Telford 2017, Ostfeld et al. 2018a). Culling deer to reduce their role as hosts for adult ticks is often mentioned as part of an integrated management plan for reducing Lyme and other tick-vectored diseases, but this approach is controversial (e.g., Kugeler et al. 2015), logistically challenging, and may not be effective unless deer densities can be maintained at fewer than approximately 5 deer per square kilometer (Telford 2017). Results from research in New England, including studies of several island communities, show that both the presence of deer and the presence of an abundant, small-mammal reservoir species are typically required for blacklegged ticks to persist and cause outbreaks of Lyme disease (e.g., Telford 2017 and references therein).

Table 2. Examples of host species, reservoir competence, and presence on Nantucket and Martha's Vineyard, including two bird species that forage on the ground.

Host species	Reservoir competence (mean percentage)	References	Presence on Nantucket	Presence on Martha's Vineyard
White-footed mouse, *Peromyscus leucopus*	92.1 93.5 62.9	LoGiudice et al. 2003, Giardina et al. 2000 Giardina et al. 2000	Present	Present
Eastern chipmunk, *Tamias striatus*	55.0 68.7 28.6	LoGiudice et al. 2003, Giardina et al. 2000 Giardina et al. 2000	Absent	Present
Short-tailed shrew, *Blarina brevicauda*	41.8	LoGiudice et al. 2003,	Present	Present
Masked shrew, *Sorex cinereus*	49.6–57.3	Brisson et al. 2008	Present	Present
Meadow vole, *Microtus pennsylvanicus*	64.0	Markowski et al. 1998 (Patience Island, RI; no white-footed mice)	Present	Present
Norway rat, *Rattus norvegicus*	61.0	Smith et al. 1993 (Monhegan Island, ME; no white-footed mice)	Present	Present
Red and gray squirrel, *Tamiascurus hudsonicus, Sciurus carolinensis*	14.7	LoGiudice et al. 2003	Present, gray squirrel	Present, gray squirrel
Striped skunk, *Mephitis mephitis*	9.7	LoGiudice et al. 2003	Absent	Present
Virginia opossum, *Didelphis virginiana*	2.6	LoGiudice et al. 2003	Absent	Absent
Raccoon, *Procyon lotor*	1.3	LoGiudice et al. 2003	Absent	Present
White-tailed deer, *Odocoileus virginianus*	4.6	LoGiudice et al. 2003	Present	Present
American Robin, *Turdus migratorius*	20.0	Giardina et al. 2000	Present	Present
Veery, *Catharus fuscescens*	28.5	Giardina et al. 2000	Absent	Rare or Absent

Note: Reservoir competence is the relative frequency of Lyme transmission from an infected host to uninfected ticks. Sources for mammal occurrences on Nantucket and Martha's Vineyard are listed in supplemental table 1. The measure of bird species occurrences is based on the Massachusetts Audubon Breeding Bird Atlas 2 (2007–2011, *www.massaudubon.org*).

New GE traits. For Mice Against Ticks, the primary GE trait will mimic an antibody-stimulating vaccine against the OspA protein (outer surface protein A) of *B. burgdorferi* that has been tested in lab mice (*Mus musculus*, Fikrig et al. 1992, Buchthal et al. 2019). A second antibody gene against the tick protein subolesin, or perhaps another tick protein, may be developed to interrupt tick feeding and disease transmission. In lab mice, Bensaci and colleagues (2012) found that vaccination with antisubolesin antibodies inhibited blacklegged tick larval infestation by 52% and reduced transmission of B. burgdorferi in surviving nymphs by 34%. Therefore, results with lab mice suggest that GE production of subolesin antibodies may reduce tick feeding and transmission in white-footed mice, although this has yet to be determined.

For the current project, the first step is to engineer white-footed mice that constitutively produce antibodies to OspA and ideally also subolesin or another tick protein, using introduced antibody genes, promoters, and enhancers that will be derived from white-footed mice (Buchthal et al. 2019). First, the research team will vaccinate lab-reared white-footed mice from a Martha's Vineyard population to identify naturally occurring antibodies that bind to OspA or subolesin. After testing the efficacy of the most promising antibodies and various types of promoters to enhance gene expression, the team plans to design a resistance cassette, with multiple antibody-encoding genes. CRISPR-based genome editing will be used to insert the resistance cassette into white-footed mouse lines, possibly with multiple copies of the resistance cassette in each mouse.

Tentative plan for field releases. Prior to any releases on Nantucket or Martha's Vineyard, smaller nearby, privately owned islands will be used in field trials (Buchthal et al. 2019). The research team also plans to initiate baseline ecological studies at all islands where GE mice would be released. Specific plans for how the first field releases will be carried out and evaluated have not been finalized, and several strategies are being considered. One possibility is to use replicated experimental plots (approximately 0.4 hectares) that are more than 500 meters (m) away from each other on same island, with two types of introductions, GE

versus non-GE white-footed mice. To promote the success of introduced mice, local mice may be trapped and removed prior to these experimental releases, whereas introduced mice could be provided with nest boxes. Little interplot movement would be expected because white-footed mice have a relatively small home range (Wolff 1985, Tsao et al. 2004), although some mice may disperse farther than 500 m (e.g., Burt 1940, Scheppe 1965). Alternatively, the research team may attempt to inundate an entire small island with GE mice, possibly in tandem with studies at an unaltered control island with no intervention and a non-GE control island with lab-reared white-footed mice that lack the GE cassette. Experimental releases could be carried out over a period of several years to increase frequencies of GE mice as needed.

Findings from the first field releases on small islands will be reported in peer-reviewed publications for the benefit of various stakeholders, scientists, funding agencies, and regulatory agencies. Data from the small-island field releases will be used to demonstrate the extent to which local white-footed mice inherit at least one copy of the GE resistance cassette within a few years. Also, quantitative, multiyear data showing declines in nymphal infection levels because of experimental introductions of GE white-footed mice will be needed to evaluate the project's ability to reduce the likelihood of Lyme transmission to humans.

If results from small-island introductions of GE mice yield promising results, scaling up to releases on Nantucket or Martha's Vineyard would be considered, pending approvals from regulatory agencies and local representatives or town meetings. The current plan is to iteratively outcross GE white-footed mice with local island populations. Once bred in very large numbers, the GE mice could be introduced either swiftly or gradually over several years. Because many residents have expressed an interest in proceeding quickly if the community as a whole approves, one plan is to release tens to hundreds of thousands of these GE white-footed mice in the early spring, when local mouse populations are their lowest level annually, in an effort to reproductively flood these populations.

Will it work?

For the purpose of this review, I assume that it is possible to use genome editing to engineer white-footed mice with resistance to the Lyme spirochete. Developing GE mouse lines that also are able to deter feeding by ticks may take longer, so I will not consider this trait for now. One can imagine many reasons why introductions of GE white-footed mice might not have the intended outcomes of reducing Lyme infections in blacklegged ticks. For example, the lab-reared GE white-footed mice might not be able to compete well with resident wild mice for resources, territories, and mating opportunities. Much will depend on the behavior and relative fitness of GE and non-GE mice and on the reservoir host use of blacklegged ticks, as is discussed below.

Even if white-footed mice with the GE resistance cassette can become established in the small-island field trials, ecological interactions on the larger islands of Nantucket and Martha's Vineyard could be complex, leading to uncertainties about how thoroughly the GE mice will be able to replace non-GE mice over time, and the extent to which populations of other reservoir host species could maintain the Lyme infection cycle on their own (Tsao et al. 2004, Brisson et al. 2008). As was noted above, several other reservoir species such as shrews, rats, robins (*Turdus migratorius*), and chipmunks (on Martha's Vineyard), might continue to infect ticks (table 2). Therefore, the combined effects of other reservoir species and any remaining non-GE white-footed mice could hinder the project's intended impact on Lyme disease. However, the research team expects that established populations of Lyme-resistant white-footed mice would deplete a major reservoir source for transmission of the spirochete, thereby significantly decreasing the proportion of Lyme-infected blacklegged ticks over time, and perhaps initiating a self-reinforcing feedback loop (personal communication, Kevin Esvelt, Massachusetts Institute of Technology, March 8, 2019, and Sam Telford III, Tufts University, March 7, 2019). Under this scenario, which merits further investigation, smaller numbers of juvenile ticks would become infected with the spirochete over time, potentially crossing a threshold level that leads to lower infection frequencies in nonmouse reservoir species.

Environmental risk assessment questions

White-footed mice are regarded as a hub species within forest communities because of their key roles as consumers of seeds, invertebrates, and fungi, and as abundant small prey for many omnivores, carnivores, and scavengers (figure 2; e.g., Ostfeld et al. 2018a). White-footed mice hoard and consume acorns, and can benefit tree species by preying on gypsy moth pupae (*Lymantria dispar*, Jones et al. 1998). In addition, these mice prey on the eggs of ground-nesting songbirds (e.g., veeries, *Catharus fuscescens*, Schmidt et al. 2006). In turn, white-footed mice are consumed by eastern screech owls (*Megascops asio*), other birds of prey, snakes (e.g., black racer, *Coluber constrictor*; milk snake, *Lampropeltis triangulum*), and various mammals (Ostfeld et al. 2018a, M. Pelikan and L. Johnson, personal communication). Dead white-footed mice also provide carrion for scavengers, including several species of burying beetle (*Nicrophorus* spp., McKenna-Foster et al. 2016). Although white-footed mice are probably too small to serve as a breeding carcass for the federally endangered American burying beetle (*Nicrophorus americanus*), which has been reintroduced on Nantucket, adult burying beetles are known to feed on dead white-footed mice (Holloway and Schnell 1997, Mckenna-Foster et al. 2016).

Like many wildlife species, white-footed mice are viewed as a pest when they cause damage in and around human habitation or serve as a vector for human diseases. Therefore, perceptions about a species' value can be highly subjective, context specific, and diverse. Given the importance of wildlife and natural areas to many of the islands' residents

Box 1. Ecological context for assessing risks and benefits of introducing GE Lyme-resistant white-footed mice in field trials and on the islands of Nantucket and Martha's Vineyard, Massachusetts (see the text for details).

Baseline conditions on each island

What are the relative contributions of local Lyme reservoir hosts (white-footed mice, shrews, and chipmunks) to the density and prevalence of Lyme-infected ticks?

Are local populations of white-footed mice distinct subspecies compared to populations on other islands and the mainland?

What is the ecological role of white-footed mice as predators, competitors, and prey? Could any rare species be affected adversely if GE interventions dramatically alter natural population dynamics of white-footed mice?

Characteristics of GE white-footed mice compared to local populations

Do GE mice differ from non-GE mice in outdoor mesocosm experiments that are designed to evaluate fitness-related traits, such as survival, growth, behavior, and reproduction?

Do introduced GE mice differ from the original populations in measures of genetic diversity?

Effects of introducing GE white-footed mice with Lyme resistance

To what extent do frequencies of Lyme-infected ticks decrease over time as was expected? If this occurs, do positive feedback loops also reduce Lyme infection levels in other reservoir species, such as masked shrews, short-tailed shrews, or chipmunks?

Does the introduction of GE white-footed mice alter the transmission dynamics of other tick-borne pathogens?

Could the introduction of GE white-footed mice conceivably lead to unwanted outcomes such as those listed in table 1?

and visitors, as well as the compelling need to reduce Lyme disease, this proposal to introduce GE Lyme-resistant white-footed mice will be of great interest to local residents and organizations such as the Massachusetts Audubon Society, the Nantucket Biodiversity Initiative, and, on Martha's Vineyard, the Wampanoag Tribe of Gay Head (Aquinnah) and BiodiversityWorks (*https://biodiversityworksmv.org*).

Many previous publications describe environmental risk assessment questions that arise whenever a new type of genetically engineered organism is developed for agriculture, forestry, aquaculture, or other applications (e.g., NAS 2002, Snow et al. 2005, Devlin et al. 2015). Similar criteria can be applied to possible risks of releasing GE white-footed mice on Nantucket and Martha's Vineyard (table 1). Independent researchers who aim to evaluate possible hazards of releasing GE white-footed mice will need detailed information about the inserted genetic elements, including DNA markers for detecting the resistance cassette, the efficacy of these genetic alterations in conferring resistance, how the introduced GE mice differ from wild mice with regard to their genetic diversity, behavior, survival, and reproduction, and how the GE mice will be released (box 1).

A key question of interest is whether GE Lyme-resistant white-footed mice will have altered fitness compared to their non-GE counterparts. Several authors have noted that white-footed mice do not seem to be harmed by spirochete infections (e.g., Schwanz et al. 2011, Voordouw et al. 2015), in which case GE mice without spirochetes may not exhibit enhanced fitness compared to infected, non-GE mice. However, empirical data to evaluate this question are scant and a field study by Ostfeld and colleagues (2018b) suggested that spirochete infection in unvaccinated white-footed mice was associated with less effective tick grooming (more ticks per mouse) and less active foraging compared to the behavior of those that were vaccinated with OspA.

Another question is whether constitutive production of resistance antibodies by GE mice could impose an underlying fitness cost, with or without spirochete infection. In addition to effects associated with engineered resistance traits per se, insertions of the resistance cassette might have unanticipated pleiotropic effects on mouse phenotypes, especially if individual mice have multiple copies of the cassette. Moreover, possible founder effects or several generations of rearing mice in captivity might result in a loss of traits that enhance their survival in the wild, including, for example, the ability to survive cold winters or years with very low acorn production.

Experiments carried out under seminatural conditions will be helpful for identifying possible fitness differences between GE and non-GE mice, although the full range of such outcomes may not be evident in small-scale, short-term studies. For example, key phenotypic differences between GE and non-GE white-footed mice might not become evident or consequential unless the mice are exposed to extreme environmental conditions, new parasites and pathogens, or certain predators or competitors. Ideally, the introduced Lyme-resistant mice would be very similar to local white-footed mice in terms of their genetic diversity, physiological ecology, behavior, fitness, population dynamics, and ecological interactions in local habitats. Although

empirical research on these topics would have to be confined to laboratory or mesocosm environments prior to planned releases, findings from such research will be helpful for identifying and evaluating possible unintended and unwanted outcomes from releasing GE Lyme-resistant white-footed mice on Nantucket, Martha's Vineyard, and other planned locations (box 1).

In formal environmental risk assessments for regulatory decision-making, researchers attempt to identify specific, measurable hazards and the likelihood of such hazards (e.g., Hayes et al. 2014, NASEM 2016, and references therein). This process represents the first phase of "problem formulation" used by regulatory agencies to evaluate GE organisms (e.g., Wolt et al. 2010). As was noted by Hayes and colleagues (2014), this very challenging task is initiated by asking many hypothetical what-if questions. For example, one could ask whether planned, massive introductions of GE white-footed mice with Lyme resistance conceivably could lead to the unwanted outcomes shown in table 1. These types of hypothetical scenarios and others may be unlikely, but it is useful to employ a problem formulation approach to identify which outcomes are deemed possible by knowledgeable experts.

Clearly, as the Mice Against Ticks project moves forward, future workshops, focus groups, and peer-reviewed publications that examine and evaluate possible environmental hazards are warranted, including studies that are carried out by independent researchers. If the MIT–Tufts team also develops an antitick cassette to prevent ticks from taking a blood meal from white-footed mice, corresponding research questions also should be evaluated. For example, if ticks are not able to feed on GE white-footed mice, information about whether this could lead to greater use of alternate hosts, including humans, or reductions in tick populations over time will be useful for evaluating possible benefits and risks.

Maintaining genetic diversity

One risk assessment concern that is relatively straightforward to address empirically pertains to population-genetic effects of releasing massive numbers of GE white-footed mice that have been reared in controlled, laboratory environments. If the native mouse lines used for rearing the GE lineages already include high levels of genetic diversity, severe bottlenecks and genetic drift can be minimized, depending on methods used for creating and maintaining these lineages. Also, white-footed mice have no close relatives with which they could hybridize on Nantucket or Martha's Vineyard, so questions about gene flow to other species via hybridization are moot.

Another consideration is whether the island populations of white-footed mice are distinct enough to merit special conservation status. Older studies in the scientific literature suggest that one or possibly two endemic subspecies of white-footed mouse may be present on Nantucket and Martha's Vineyard (Waters 1969, Argyros 2004). However, differentiation among the island and mainland populations may be relatively small given the large amount of genetic, morphological, and behavioral variation found in this species across its range (Bedford and Hoekstra 2015). Resampling island and mainland populations using modern approaches such as whole genome sequencing combined with phenotypic data would be a useful starting point for reevaluating the extent of genetic divergence among these groups, as well as genetic diversity within populations (Ellegren 2014, Coates et al. 2018). Within-population data could then be used to compare genetic profiles of current populations with subsequent populations after massive introductions of GE white-footed mice on Nantucket and Martha's Vineyard.

How will genome-edited white-footed mice be regulated?

At the state level, the Massachusetts Division of Fisheries and Wildlife regulates the release of all wildlife species regardless of their GE status (*www.mass.gov*). Specifically, the Code of Massachusetts Regulations (321 CMR 2.15[4]) states that a permit is required to release wild animals on the basis of stipulations of the state's wildlife management practices. In addition, Massachusetts Division of Fisheries and Wildlife may require that the genetic uniqueness of island populations on Nantucket and Martha's Vineyard be preserved (personal communication, Thomas French, Massachusetts Division of Fisheries and Wildlife, 18 February 2019). Therefore, separate GE founding populations representing the genetic diversity found on each island would need to be developed.

At the federal level, many possible uses of CRISPR-based genome editing are not subject to clear or consistent regulatory oversight (Kuzma 2016, NASEM 2017, Brossard et al. 2019). To my knowledge, this would be the first application of genetic engineering for the intentional, uncontained release of a wild mammal in the United States or elsewhere. To date, the US Food and Drug Administration (FDA) has regulated transgenic fish and mosquitoes on the basis of the 1986 US Coordinated Framework for the Regulation of Biotechnology (NASEM 2016, 2017). Under FDA's draft guidance for industry no.187, revised in January 2017, the agency regulates "intentionally altered genomic DNA in animals," including the use of CRISPR, under the process for evaluating "new animal drugs" (e.g., *www.fda.gov*). FDA guidance no.187 states that discretionary regulation is based on asking "does the animal with intentionally altered genomic DNA pose any more of an environmental risk than its counterpart?" How this question is addressed and whether a formal environmental impact statement is required under NEPA (National Environmental Policy Act) appear to be case dependent (*www.fda.gov*, Meghani and Kuzma 2017). The Environmental Protection Agency also could become involved if the GE white-footed mice are endowed with an antitick cassette and if this is viewed as a type of "pesticide" (NASEM 2017).

On the basis of my informal inquiries, federal agencies appear to be waiting for a concrete application to be submitted

before they are able to identify which regulations and procedures would apply. This delay makes it challenging for researchers, NGOs, and others to know what kinds of specific baseline data should be obtained to address regulatory requirements for field trials and larger-scale releases of GE vertebrates.

Conclusions

Although the Mice Against Ticks project is still at an early stage of development, Esvelt's team has publicized their intial methods and long-term goals well in advance to encourage early involvement and oversight from various stakeholders (Buchthal et al. 2019). Their decision to do so provides a unique opportunity for professional ecologists and others to offer advice and contribute data that can be used to evaluate and address possible risks such as those described in the present article. Because the earliest, small-scale field trials with GE white-footed mice are not likely to take place in the next 3–5 years, at the earliest, or more likely several years later because of engineering difficulty, coordinated research can be initiated soon to address key questions related to risk assessment.

Several research questions posed in the present article would be highly suitable for graduate students' theses and dissertations, whereas others are more feasible for the MIT–Tufts group, other research teams, or professionals who work with citizen-science collaborators. Not every ecological or evolutionary question raised in the present article can be answered with empirical data, in which case modeling studies and extensive knowledge of relevant topics will be needed. In summary, a combination of scientific literature review, new studies to address research gaps, and the application of formal risk assessment protocols can be used to examine this case study from an environmental standpoint. With this information, possible risks can be weighed against possible benefits to society—that is, greatly decreasing Lyme disease exposure on Nantucket and Martha's Vineyard.

In the near future, many other novel and more controversial applications of CRISPR-based genome editing in wild vertebrates are likely to be proposed, including the use of gene drives coupled with sterility genes to eradicate invasive pest populations (NASEM 2016, Harvey-Samuel et al. 2017, Piaggio et al. 2017). Meanwhile, gene drive research to eradicate mosquito populations that carry malaria is already well underway (Kyrou et al. 2018). Kevin Esvelt's group and others are working on nonglobal gene drives and other self-limiting approaches that are expected to be safer than global gene drives (Noble et al. 2016). However, possible long-term consequences of releasing wild animals gene drives are not well understood (Webber et al. 2015, NASEM 2017, Dhole et al. 2018) and the absence of harmonized, international oversight for environmental biosafety and public engagement is a major concern (Kofler et al. 2018). Going forward, interdisciplinary cooperation and proactive risk assessment research will be needed to identify appropriate uses of genome editing in wild vertebrates and to avoid unwanted outcomes.

Acknowledgments

I thank Kevin Esvelt, Sam Telford III, and Joanna Buchthal for posting information about this project on their website (*www.responsivescience.org*) and checking this description of their project for accuracy; Thomas French, Lisle Gibbs, Emily Goldstein, John Harder, Luanne Johnson, Hans Klompen, Jennifer Kuzma, Taal Levi, Philip Myers, Richard Ostfeld, Matthew Pelican, Werner Schenkel, and several anonymous reviewers provided helpful comments on the manuscript.

Supplemental material

Supplemental data are available at *BIOSCI* online.

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