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Combs, Matthew A.

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Genetic Tools and Ecological Context for Commensal Rodent Management: Population Genetic Analysis and Gene Drives

Matthew A. Combs

USDA APHIS Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado

ABSTRACT: Invasive commensal rodents are the source of significant harms across diverse ecosystems globally, including biodiversity loss on islands, economic damage in agricultural settings, and disease spread in urban areas. Genetic tools can provide unique insights and solutions for rodent management or eradication, adding to the toolbox of integrated pest management operations. In real world conditions, the utility of genetic tools for rodent management is constrained by ecological factors such as mating and dispersal behavior, landscape variation, and ongoing management strategies. Here we describe two distinct uses of genetic tools for rodent management and their ecological considerations. First, we describe how patterns of genetic connectivity can inform the establishment of functional management units, discern among scenarios leading to island eradication failure, and provide insights into rodent management outcomes. Second, we discuss the utility and risks of using gene drive systems to eradicate invasive house mice from island environments. We describe aspects of risk assessment and mitigation as well as the utility of computational models for improving gene drive preparedness. Together, we outline the importance of ecologically-informed implementation strategies when using genetic tools, both currently available and in development, for the management of commensal rodents.

KEY WORDS: eradication, gene drive, house mouse, integrated pest management, *Mus musculus*, population genetics, rodents

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INTRODUCTION

Commensal rodents, including species such as the house mouse (*Mus musculus*), brown rat (*Rattus norvegicus*), and black rat (*R. rattus*), are a major source of environmental damages worldwide (Pimentel et al. 2005, Towns et al. 2006). These species pose a considerable threat to biodiversity, impacting threatened and endangered species on islands (Harris 2009, St Clair 2011). In human settlements, commensal rodents generate huge economic burdens through impacts on goods and infrastructure (Pimentel et al. 2005). Additionally, these species are important hosts of many zoonotic pathogens in urban and non-urban environments, posing a threat to public health when spillover events lead to human disease (Combs et al. 2021, Plowright et al. 2017). Rodent management campaigns are designed to limit the impacts of these damages by reducing or eradicating local populations using a variety of strategies and technologies.

The pest management toolbox for commensal rodents has continued to expand and adapt over the past century with the introduction of new tools and strategies. Integrated pest management (IPM) approaches have encouraged managers to think holistically about the ecology of rodent populations and limit the use of rodenticides when possible (DeVault et al. 1996, Dhawan and Peshin 2009). IPM strategies include habitat modifications, education, reproductive control technologies, and strategic timing and placement of toxicants, among others. Modern toxicants such as second-generation anticoagulants have also shown improvements by limiting avoidance behaviors and reducing the likelihood and impact of evolved toxicant resistance (Berny et al. 2018). Tracking technologies, spatial analyses, and statistical inferences have also provided unique insights into the movement and habitat

selection behaviors of rodents across a variety of landscapes (Byers et al. 2017, Traweger et al. 2006, van Adrichem et al. 2013). Indeed, the adoption of new research strategies and the willingness to work with biologists for unique insights are key strengths of modern rodent management science and programs.

Genetic technologies and analyses have the capacity to provide additional tools within the pest management toolbox. Here we discuss two distinct uses of genetic tools applicable across different sectors of commensal rodent management. First, we discuss how population genetic patterns can be leveraged to gain insights into critical biological processes and to inform location-specific management strategies. Second, we outline the utility of engineered gene drives for targeted population suppression. While population genetic tools are currently available and in use across the world, gene drive technologies are still in development. We discuss the utility and practical implications of both approaches to help expand the toolbox of pest managers.

POPULATION GENETICS FOR MANAGEMENT INSIGHTS

Population genetics describes patterns of genetic diversity and similarity among individuals or groups of individuals and their changes over time (Gillespie 2004). These patterns are structured in part by the behaviors of species as well as the ecological conditions and evolutionary pressures they experience. Thus, we can use population genetic patterns to understand aspects of dispersal and population dynamics that can ultimately inform pest management strategies. Generally, pest management seeks to reduce or remove target populations and prevent future damages by limiting the capacity for remnant

populations to rebound, or for new individuals to immigrate into treated areas and re-establish new populations. Below we describe three different possible uses of population genetics related to informing management units, understanding invasion and re-invasion pressure, and capturing evidence of management outcomes.

An important and directly applicable result of population genetic analysis of commensal rodent management areas is the delineation of distinct genetic populations or clusters, which can be used to define management units. To understand this application, it is important to understand how movement behaviors and population dynamics generation population genetic structure. The extent of population genetic differentiation at neutral loci is determined by the opposing evolutionary forces of genetic drift and gene flow. Genetic drift acts through the random loss of alleles, which leads to decreasing diversity and increasing differentiation to other populations (Slatkin 1987). Gene flow occurs when individuals migrate between populations and reproduce, leading to increased genetic similarity between populations (Slatkin 1987). Genetic populations are expected to exhibit more migration within the geographic bounds of that population than among neighboring populations. Genetic populations are often separated by migration barriers, which may be landscape features that are difficult to migrate across due to behavioral or physiological constraints. Thus, the boundaries of genetic populations can be used to inform eradication or management units with the expectation that the likelihood of rapid reinvasion is relatively lower when individuals have to cross a migration barrier (Combs et al. 2019). Achieving this management insight relies on appropriate sampling of individuals or local groups across the entire spatial distribution of the targeted population (Peterman et al. 2016).

Genetic populations and migration barriers for rodents have been identified at a variety of scales, including within individual neighborhoods (Combs et al. 2018), across cities (Badou et al. 2021, Combs et al. 2017), and globally (Puckett et al. 2016). While the presence of migration barriers implies reduced migration pressure following rodent management campaigns, it is important to recognize that social and territorial behaviors can play a role in preventing migrants from successfully establishing (Barnett and Spencer 1951). Removing extant populations also removes those social barriers so rapid reinvasion and establishment is still possible, particularly when abundant resources and harborage exist in treated areas (Barnett and Spencer 1951, Fraser et al. 2014).

Population genetics can provide unique insights into the processes that lead to eradication failure. When rodents are detected months or years after management efforts, particularly for island eradication campaigns, the key question is whether rodent populations were never completely eradicated and rebounded naturally from remnant individuals, or whether rodents reinvaded from nearby locations. By acquiring genetic samples from populations before management efforts begin, as well as from multiple potential source populations, the origin of newly emerging rodents can be easily determined through

population genetic analysis. For example, an analysis on the Bischof islands of Haida Gwaii, British Columbia, Canada determined that rats identified after management efforts were likely due to migration from nearby Lyell Island rather than survivors of the original Bischof Islands population (Sjodin et al. 2020). Population genetic insights such as these help managers to develop insight into strategies that improve long term island eradication outcomes.

Genetic analysis can also be useful for observing the impacts of management outcomes on treated populations. While direct abundance estimates through the use of detector baits, camera traps, or tracking pads can often be effective and cost efficient, genetic estimations of effective population size can also be used to infer changes in abundance. Effective population size reflects the number of individuals contributing to a population's overall evolutionary and demographic trajectory, and is different than the true abundance or census size (Frankham 1995). One recent study using this approach identified clear decreases in effective population size among rats in multiple treated neighborhoods in Salvador, Brazil (Richardson et al. 2019). Effective population size can also be an indicator of the overall adaptive potential of populations, with relevance to the likelihood of rodenticide resistance arising. It is important to recognize though, that evolutionary changes reflected in population genetic signatures often occur with a time lag from the events that altered populations. Thus, changes in effective population size may not directly reflect relative changes to a population's census size for several generations (Frankham 1995). In the same way, unique genetic populations may still be identified long after the removal of a migration barrier, until sufficient gene flow occurs to homogenize populations.

Overall, population genetic analysis can provide unique insights to inform management strategies and outcomes. Still, it is important to recognize that the cost of genetic sequencing and the technical skills required to analyze genetic data can be barriers for rodent management projects. Fortunately, advances in sequencing technology and library preparation continue to decrease the cost per sequencing read and informed sampling approaches can allow for actionable insights with samples from small numbers of individuals (Rollins et al. 2006). Collaborations between pest managers, academics, government agencies, and non-profits can help to make genetic analysis more accessible and affordable when expertise and resources can be distributed among several cooperating entities.

ENGINEERED GENE DRIVES FOR DIRECTED POPULATION SUPPRESSION

Gene drives are genetic systems that positively skew the inheritance of specific alleles (i.e. super-mendelian inheritance), causing them to rapidly increase in population frequency (Alphey et al. 2020). Engineered "suppression" gene drives are designed to eradicate populations by skewing the sex-ratio of offspring, altering fertility, or causing lethality in specific genotypes (Bier 2022). Thus,

gene drives may offer an alternative tool for managing populations that use genetics to directly alter the genotypes of populations and drive those populations to local extinction (Campbell et al. 2019). One potential advantage of gene drives over conventional toxicants for rodent suppression is that they leverage the mate finding and movement behaviors to spread through populations. Ideally, these behaviors allow gene drives to impact every individual and avoiding population rebound from a small number of survivors, as has been observed following toxicant deployments intended to eradicate islands (Howald et al. 2007). While suppression gene drives for rodents are currently under development, to date none have been tested in field trials or approved for release into natural environments (Gierus et al. 2022, Wells and Steinbrecher 2023).

Many different gene drive strategies have been proposed and developed in laboratories (Wells and Steinbrecher 2023). Each gene drive strategy has different constraints and advantages that impact the required number of released individuals, the spatial spread and reversibility, time required for eradication, and the overall likelihood of success (Bier 2022). Low-threshold gene drives are expected to spread rapidly and continuously through populations with the introduction of even a small number of gene drive modified organisms, such as the X-Shredder or driving-Y drive (Deredec et al. 2008). Other gene drives, known as high-threshold gene drives, must be released in much higher abundance in order to spread through populations and are expected to be more spatially limited, as their relative abundance decreases towards the outer edges of the gene drive wave (Deredec et al. 2008). *Medea* toxin-antidote systems are an example of high-threshold gene drives (Akbari et al. 2014, Champer et al. 2016).

Gene drives are novel biotechnologies that are not without potential risks to the environment, human health, or local and international economies (Devos et al. 2021, Hayes et al. 2018). It is critical that quantitative risk assessments help evaluate the likelihood of potential harms and facilitate responsible decision making. While strong regulatory frameworks exist for the evaluation of other genetically modified organisms, gene drives pose unique challenges for risk assessors because they can evolve over time, spread over vast spatial areas, and potentially persist in wild populations for many generations (National Academies of Sciences, Engineering, and Medicine et al. 2016). Often, risk assessments are informed through empirical data from limited field trials, though such field trials for gene drives may not be possible given the potential for animals to actively escape confinement. Given the difficulty of obtaining empirical data on gene drive dynamics, computational models are expected to provide crucial insights into the potential risks and outcomes of introduced gene drives (Combs et al. 2023, Golnar et al. 2021).

Given the considerable risk of undesirable spread to non-target populations or geographic areas, many gene drive designs are engineered to reduce the likelihood of environmental risks. For example, gene drives targeting

locally fixed alleles are expected to function only in their target population which would harbor a geographically unique genetic sequence recognized by the gene drive system (Sudweeks et al. 2019). Split drive systems use physical separation of gene drive molecular components, such that the drive system operates correctly only when multiple components are inherited independently, which could reduce the likelihood of transmission into non-target populations in the event of gene drive organisms escaping confinement during trials (Akbari et al. 2015). Chemically reversible gene drives have even been designed, which use the introduction of a non-toxic chemical signal in the environment to break the functional capacity of a gene drive system, effectively limiting future spread (Akbari et al. 2013, Chae et al. 2020). The development of gene drive technologies focused on environmental safety and risk mitigation will likely be a critical avenue of research for the realization of gene drives as a tool for rodent management.

In addition to the need for risk assessment and regulatory frameworks, the field of gene drives must overcome several practical hurdles before they may become a realistic tool for rodent managers. Gene drive systems in rodents must operate at very high efficiencies to effectively spread through populations and avoid the evolution of resistant alleles (Carrami et al. 2018). Though recent advances in the t-CRISPR gene drive system in house mice shows promise, mammalian gene drives have historically been much more difficult to successfully develop than those designed for certain mosquito species (Gierus et al. 2022, Pfitzner et al. 2020). Ecological characteristics of populations may also prevent successful eradications even when drive systems function at high efficiencies (Kim et al. 2023). For example, polyandry, which is common in rodent populations, can limit the spread of gene drives when wild-type individuals maintain a fitness advantage over gene-drive harboring individuals (Manser et al. 2020). The spatial dynamics of rodent movement behavior can also serve to limit the effectiveness of gene drives when wild-type individuals re-invade previously eradicated areas, leading to unstable waves of localized population growth and crashing known as “chasing” dynamics (Champer et al. 2021). Further, it is unclear whether gene drives will obtain a “social license” for introduction into wild populations from either locally impacted human communities or at national and international levels. Dedicated engagement efforts will be required to understand and respond to the concerns of people living in areas where gene drives are introduced or that have strong economic or cultural connections to those landscapes (Godwin et al. 2019, Kokotovich et al. 2022).

CONCLUSION

Commensal rodents are notoriously difficult to manage due to their high fecundity, generalist ecology, and often cryptic distributions. Harnessing tools from the field of population genetics can provide novel insights and applications to combat commensal rodents across a variety of habitats, from uninhabited tropical islands to

densely populated urban centers. Here we have discussed the utility of two distinct uses of genetic tools within the field of rodent management, leveraging population genetic analysis for improved management strategies and evaluation as well as the potential promise of suppression gene drives for eradicating local rodent populations. While population genetic analyses have become increasingly adopted within rodent management studies, the development of gene drive technologies and regulatory frameworks require significant advancement before they are a realistic option for managers. Together these approaches reflect a partial snapshot of the utility of genetic tools within the field of commensal rodent management, their capacity to improve outcomes, and the potential for advancement with continued research and application.

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