Ecological Applications Topic: **Using Self-Replicating Gene Drives That Can Spread Deleterious Alleles Through Animal Populations on Islands**

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Invasive species are a primary threat to native biodiversity on islands and it is agreed upon worldwide that eradicating invasive species is a mainstream idea for practicing conservation. (7) One technique that is being studied is using gene drive to spread deleterious alleles and control invasive species. An example of this using is CRISPR/Cas12a to perform a Y chromosome deletion on mice, known as Y-CHOPE, targeting the 37 A, 37 B, and 59 sites (1, 2). Once an RNA-guided cut is performed the desired result is homologous recombination. Therefore, all heterozygote males will change to homozygotes and become XO-genotype females when they develop during birth. Although this sounds ideal it has been found that, especially on island, this technique can create resistance through nonhomologous end joining or not even be effective at all. This happens when the cells repair itself imperfectly by ligating the two cut ends together and produces a mutation. (1, 5, & 6) Not only do you have to consider these factors, but researchers have also found there are other components that effect gene drive such as environment, embryonic resistance rate, etc. (3, 4, & 6) Progress has been made to prove that we can drive conversion efficiency and reduce theses germline resistance rates but more research must be performed. (1,3, & 6).

Annotated Bibliography

All sources were found on PubMed and were found in the "similar article" section of article number 1.

1. Prowse, T., Cassey, P., Ross, J. V., Pfitzner, C., Wittmann, T. A., & Thomas, P. (2017). Dodging silver bullets: good CRISPR gene-drive design is critical for eradicating exotic vertebrates. *Proceedings. Biological sciences*, *284*(1860), 20170799. doi:10.1098/rspb.2017.0799 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5563802/#

This research article discusses the use of self-replicating genes that can spread deleterious alleles. This technique is being used to control invasive species especially those in island populations. The authors discuss the efficacy of this technique since we know that the target organism can create a resistance to this method through nonhomologous end joining. They studied multiple types of gene drive techniques and ultimately found that it was necessary to multiplex gRNA to ensure that the gene drive has a low risk and high probabilities of eradicating an invasive species. Especially island populations.

2. Prowse, T. A., Adikusuma, F., Cassey, P., Thomas, P., & Ross, J. V. (2019). A Y-chromosome shredding gene drive for controlling pest vertebrate populations. *eLife*, *8*, e41873. doi:10.7554/eLife.41873 https://www.ncbi.nlm.nih.gov/pubmed/30767891

This research article focused on the "Y-CHOPE" method of gene drive using CRISPR to target the Y chromosome and delete it. This causes XY males to become XO fertile females. They tested this method on stem cells with a 90% success rate and then repeated this process with mice. Their conclusion states that Y-CHOPE is a viable method for pest control.

3. Champer, J., Liu, J., Oh, S. Y., Reeves, R., Luthra, A., Oakes, N., … Messer, P. W. (2018). Reducing resistance allele formation in CRISPR gene drive. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(21), 5522–5527. doi:10.1073/pnas.1720354115 https://www.ncbi.nlm.nih.gov/pubmed/29735716

This research article discusses the possibilities of controlling vector borne disease through gene drive and how multiplexing gRNAs can both significantly increase the drive conversion efficiency and reduce germline resistance rates of a CRISPR homing gene drive in *Drosophila melanogaster*. The researchers were not only able to create a gene drive with no subsequent resistance, but they were also to distinguish which promoter is the best option for this type of strategy. Ultimately, the authors results showed that drive conversion and germline resistance rates are similar between different genomic targets, while embryo resistance rates can vary significantly.

4. McCreless, E. E., Huff, D. D., Croll, D. A., Tershy, B. R., Spatz, D. R., Holmes, N. D., … Wilcox, C. (2016). Past and estimated future impact of invasive alien mammals on insular threatened vertebrate populations. *Nature communications*, *7*, 12488. doi:10.1038/ncomms12488 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4992154/

In this study the authors discuss the importance of the threat that invasive mammals pose to island populations. They do so by modelling global extirpation patterns for island populations of threatened and extinct vertebrates. Using the data from many different species, island sizes, etc. the authors were able to show that controlling or eradicating the relevant invasive mammals could prevent 41-75% of predicted future extirpations. The authors also concluded that eradicating these invasive mammals will have much greater on smaller, dryer islands than large, wet islands.

5. Oberhofer, G., Ivy, T., & Hay, B. A. (2018). Behavior of homing endonuclease gene drives targeting genes required for viability or female fertility with multiplexed guide RNAs. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(40), E9343–E9352. doi:10.1073/pnas.1805278115 https://www.ncbi.nlm.nih.gov/pubmed/30224454

This study focuses on homing endonuclease gene (HEG), how they bring about population suppression, and how this creates alleles that are resistant to cleavage but retain wild-type function. They show that this can be prevented through the use of gRNA but it led to other complications that prevented drive. The use this research to propose strategies to overcome these issues for next-generation HEG systems.

6. Pest demography critically determines the viability of synthetic gene drives for population control. (2018, September 13). Retrieved April 2, 2019, from https://www.sciencedirect.com/science/article/abs/pii/S0025556418301172?via=ihub

This research article talks about gene drive and how it is a solution to controlling invasive species. What this study focuses on is collecting data based on hypothetical pest demography and how it impacts the expected progression of 2 gene drive strategies through populations. Their results showed that mass-action reproduction results in overly optimistic eradication while polygyny reproduction failed due to evolution of resistance unless a reproductive Allee (reduced reproductive rates at low population density) effect was also included. These results did not change significantly when the size of the initial gene-drive was increased. These results show that demography is critical when determining viability of gene-drive suppression.

7. Dawson, J., Oppel, S., Cuthbert, R. J., Holmes, N., Bird, J. P., Butchart, S. H., . . . Tershy, B. (2015, February). Prioritizing islands for the eradication of invasive vertebrates in the United Kingdom overseas territories. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25163543

In this study it is stated that invasive species are a primary threat to native biodiversity on islands. This is accepting that eradicating invasive species is a mainstream conservation practice and its goal is to create a list prioritizing which islands, from 11 U.K. overseas territories, that require the most attention. This based off eradication feasibility and the benefit of the eradication of the invasive species. This approach can be repeated and duplicated anywhere in the world

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