#978: Overcoming Evolved Resistance to Population-suppressing Homing-based Gene Drives

John Marshall[1], Anna Buchman[2], Héctor M. Sánchez C.[3], Omar S. Akbari[2]

[1]Divisions of Biostatistics and Epidemiology, School of Public Health, University of California, Berkeley, California (john.marshall@berkeley.edu). [2]Department of Entomology, Center for Disease Vector Research, University of California, Riverside, California. [3]Bioinformatics Research Group, School of Medicine, Tecnológico de Monterrey, Estado de México, México.

ABSTRACT

The recent development of a CRISPR-Cas9-based homing system for the suppression of *Anopheles gambiae* is encouraging; however, with current designs, the emergence of homing-resistant alleles is expected to result in suppressed populations rapidly rebounding, as homing-resistant alleles have a significant fitness advantage over functional homing alleles. We develop a mathematical model to estimate tolerable rates of homing-resistant allele generation to suppress a wild population of a given size. Our results suggest that, to achieve meaningful population suppression, tolerable rates of resistance allele generation are orders of magnitude smaller than those observed. To remedy this, we propose an architecture in which guide RNAs are multiplexed. Modeling results suggest that the size of the population that can be suppressed increases exponentially with the number of multiplexed gRNAs and that, with six multiplexed gRNAs, a mosquito species could potentially be suppressed on a continental scale.

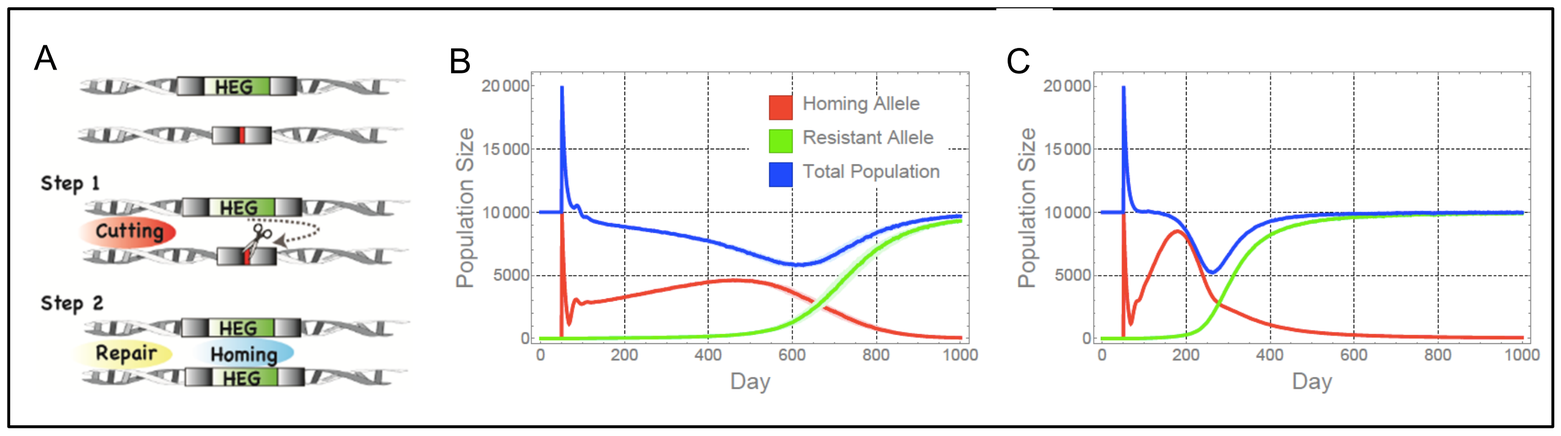

Figure 1. Predicted population dynamics for current CRISPR-Cas9 population suppression homing constructs. (A) Homing constructs spread by cleaving the homologous chromosome at a given target site and using the homology-directed repair (HDR) pathway to repair the cleaved chromosome with the one carrying the homing construct. Here, we model a population suppression homing construct with a homing rate of 98% and a resistant allele generation rate of 1% in a population of 10,000 adults. Red lines represent individuals having at least one copy of the homing allele, green lines represent individuals having at least one copy of the homing-resistant allele, and blue lines represent the total population. In panel (B), dynamics are shown for the scenario in which females heterozygous for the homing allele have their fertility reduced by 90.7%. In panel (C), the same construct is modeled in the absence of a fertility cost. In both cases, population suppression is moderate and short-lived.

LARGER POPULATIONS CAN BE SUPPRESSED WITH SMALLER RESISTANT ALLELE GENERATION RATES

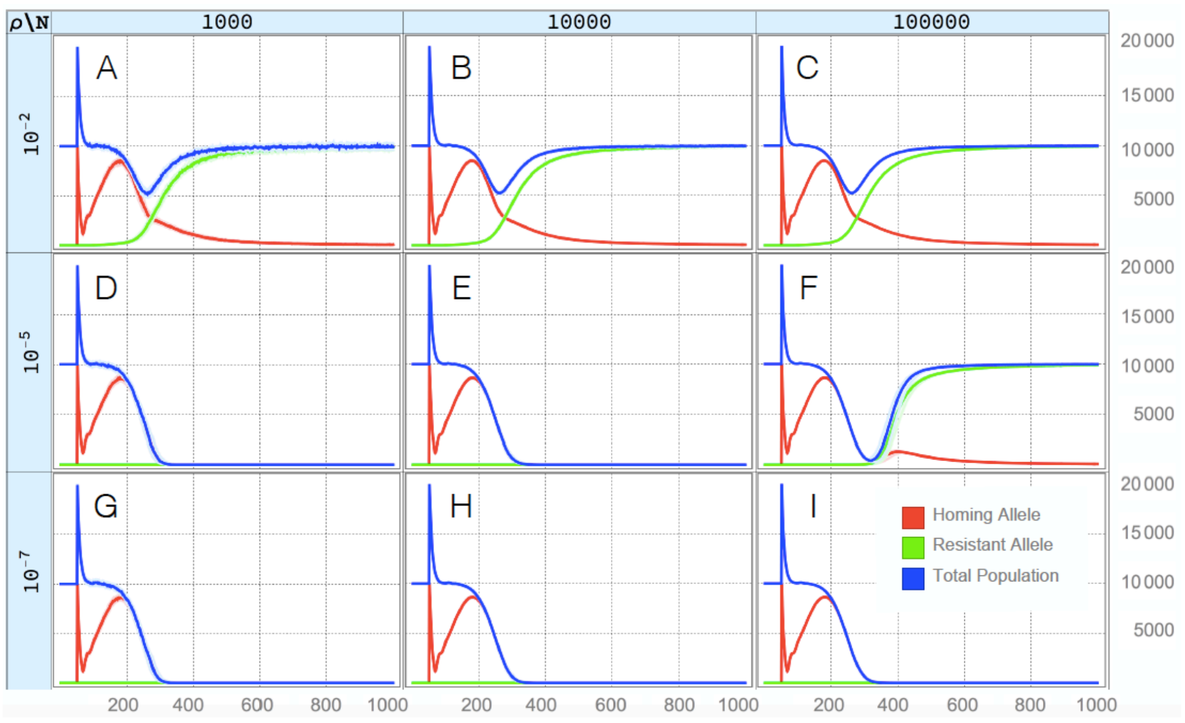

Figure 2. Here, we model a population suppression homing construct with a homing rate of 98% and no fertility cost. In panels (A-C) the resistant allele generation rate is 1% (10^{-2}), in panels (D-F) it is 0.001% (10^{-5}), and in panels (G-I) it is 0.00001% (10^{-7}). In the leftmost panels (A, D and G), a population of 1,000 is modeled, in the middle panels (B, E and H), it is 10,000, and in the rightmost panels (C, F and I), it is 100,000. As the resistant allele generation rate is reduced, we expect to be able to eliminate populations of larger sizes.

POPULATION ELIMINATION IS HIGHLY DEPENDENT ON THE RESISTANT ALLELE GENERATION RATE BUT NOT ON THE HOMING RATE

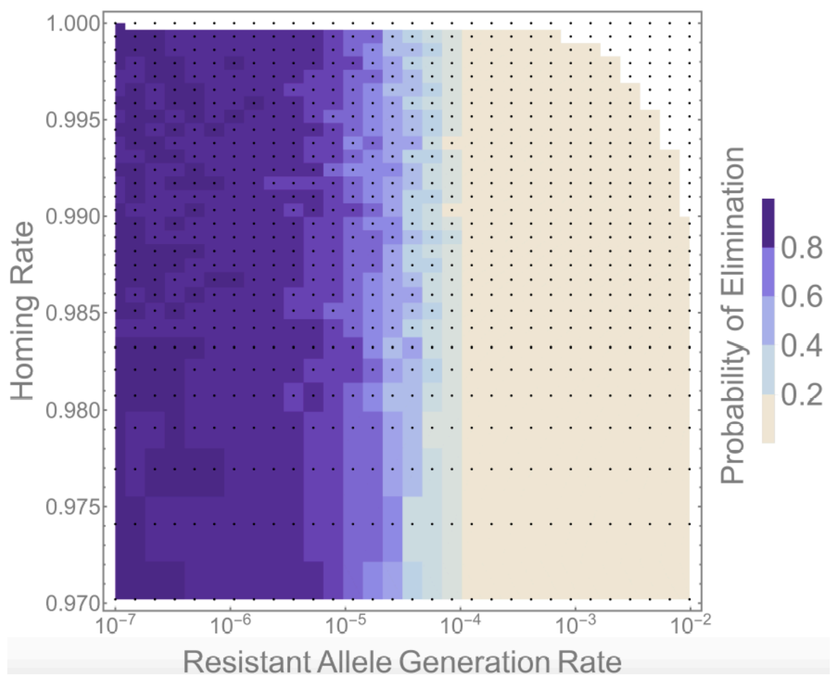

Figure 3. Here, we model a population suppression homing construct in a population of 10,000 adult mosquitoes. Each pixel represents a combination of homing and resistant allele generation rates for which the simulation was run. Pixel shadings represent the proportion of 100 simulations in which population elimination was achieved. Population elimination probability is independent of the homing rate (for already high homing rates) and critically dependent on the resistant allele generation rate.

DESIGN CRITERIA FOR RESISTANT ALLELE GENERATION RATE / MULTIPLEX NUMBER

The linear relationship between $1/N$ and the resistant allele generation rate (ρ) leads to the following design criteria for multiplex number (m) required to have a 95% probability of eliminating a population of size N: $m > (\log 0.0199 - \log N) / \log \rho$

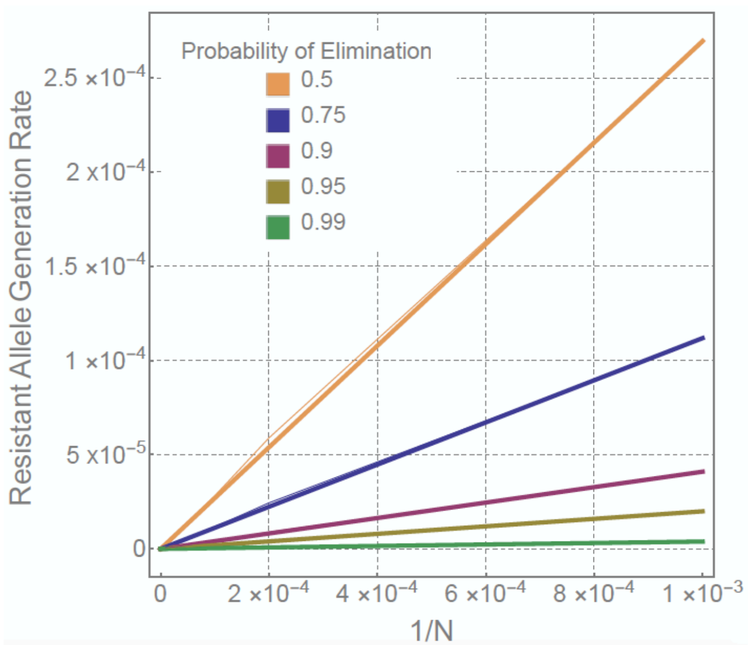

Figure 4. Linear relationship between $1/N$ and the resistant allele generation rate leading to a given probability of population elimination, as determined by a series of model simulations as described in Figure 3.

SUCCESSFUL DEMONSTRACTION OF MULTIPLEXING IN DROSOPHILA MELANOGASTER

A

B

Parental Genotypes	*gRNA#1 white (w)*	*gRNA#2 yellow(y)*
♀ *yw; RGR/RGR* X ♂ *yw; Vasa-Cas9/Vasa-Cas9*	100 +/- 0% (1053/1053)	60.5 +/- 0.43% (637/1053)
♂ *yw; RGR/RGR* X ♀ *yw; Vasa-Cas9/Vasa-Cas9*	100 +/- 0% (712/712)	87 +/- 0.41% (616/712)

Figure 5. (A) Schematic of the OA-16 construct used to demonstrate multiplexing. The first and second gRNAs (targeting white and yellow, respectively) are shown in grey. Each gRNA has a hammerhead ribozyme 5' (shown in blue) and an HDV ribozyme 3' (shown in green). The gRNAs are driven by a single Drosophila ubiquitin polII promoter. (B) Crossing scheme used to generate mutants and obtained results. Individual male and female flies homozygous for the OA-16 construct were crossed to individual female and male flies, respectively, of a homozygous vasa-Cas9 line. Progeny were scored for eye and body color. Percentages correspond to number of flies out of total cross progeny exhibiting a mutation for each gRNA.

ACKNOWLEDGEMENTS

This work was supported by a UC MEXUS grant (CN-15-47) awarded to J.M.M., a gift from The Parker Foundation to the Malaria Elimination Initiative at UC San Francisco, UC Riverside laboratory start-up funds awarded to O.S.A., a private donation by MaxMind awarded to O.S.A., and a US National Institutes of Health (NIH) K22 grant (5K22AI113060-02) awarded to O.S.A.