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GENETIC ENGINEERING

On the road to efficient gene drives

Containment of malaria transmission largely depends on vector control — that is, on eliminating the parasite-transmitting *Anopheles* mosquito — and gene drives have emerged as a promising approach to achieve this goal. In a new study published in *PLoS Genetics*, Hammond *et al.* address one of the major problems associated with the gene drive technology: the accumulation of mutations that drive resistance to the gene drive in the mosquito population.

Because of their selfish nature, gene drives inserted into one allele can — through the activity of their encoded nuclease — integrate into the homologous chromosome, thereby favouring their own inheritance. The CRISPR–Cas9-based gene drive used in the current work specifically targets and disrupts a haplosufficient mosquito gene required for female fertility. Thus, as it spreads across the mosquito population, the gene drive reduces reproductive potential.

As expected and known from published work, female mosquitoes harbouring the gene drive initially showed a sharp drop in fertility, consistent with the successful propagation of the gene drive in the population. However, after 25 generations, the gene drive was present in only 20% of individuals. Sequencing of the gene drive target sites revealed the accumulation of short in-frame insertions and deletions (indels) that restored target gene functionality while making the target site resistant to further cleavage.

Crossing generation-20 females with wild-type males produced viable and fertile offspring; however, the inheritance rate of the gene drive suggested normal Mendelian segregation, that is, resistance to the gene drive element.

To assess whether the gene drive was still functional, the team crossed male offspring ('sons') with wild-type females — that is, with mosquitos harbouring a non-mutated gene drive target site. Although gene drive transmission to offspring increased significantly, indicating an intact nuclease, the rate of transmission (the 'homing' efficiency) was reduced to an average of 60% compared with 99% in previous observations.

The authors posit that the reduced gene drive activity in sons may arise because fertilized embryos contain remaining maternal Cas9 activity that leads to the repair of DNA double-strand breaks by end joining instead of homologous recombination. Consistent with this notion, 'grandsons' — which harbour only a paternal gene drive copy — showed again highly efficient homing (97.5%).

Finally, the authors discuss how gene drives can be designed more cleverly to prevent resistance. Suggested approaches include simultaneously aiming at multiple target sites, selection of target sites that show low tolerance towards sequence variation and better maternal promoter control to prevent deposition of residual nuclease in the embryo. Taken together, these considerations bring us a step further towards the design of efficient and safe gene drives.

Michelle Trenkmann, Associate Editor,
Nature Communications

> "as it spreads across the mosquito population, the gene drive reduces reproductive potential"

ORIGINAL ARTICLE Hammond, A. M. *et al.* The creation and selection of mutations resistant to a gene drive over multiple generations in the malaria mosquito. *PLoS Genet.* http://dx.doi.org/10.1371/journal.pgen.1007039 (2017)
FURTHER READING Champer, J. *et al.* Cheating evolution: engineering gene drives to manipulate the fate of wild populations. *Nat. Rev. Genet.* **17**, 146–159 (2016)

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