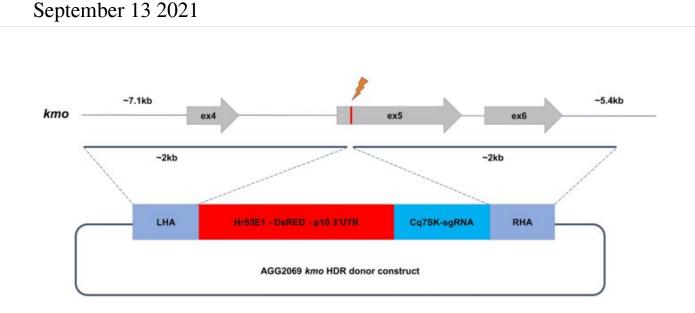


Scientists use gene editing tool to target mosquito-spread disease



CRISPR/Cas9 based kmo knock-in cassette. Representation of the kmo locus and HDR donor construct for integration. Grey arrows represent exons and the red vertical line indicates the sgRNA target site within exon 5. The dark gray lines indicate the left and right homology arm sequences (LHA and RHA, respectively). In addition to a fluorescent marker, the knock-in cassette contains the C. quinquefasciatus 7SK promoter, an RNA Pol III promoter which expresses the same sgRNA used to integrate the cassette into kmo. This would allow an integration of this cassette to be combined with future cas9 expressing lines to test for homing activity. Credit: DOI: 10.1038/s41598-021-94065-z

A new gene editing tool has been successfully applied to the southern house mosquito by researchers at the BBSRC-funded Pirbright Institute.



This paves the way for genetic control methods that could prevent the mosquito from spreading human and animal diseases.

Targeting the southern house mosquito

The female southern <u>house</u> mosquito (Culex quinquefasciatus say) largely feeds on birds, which results in the transmission of avian malaria, an important contributor to the extinction of several avian species.

This mosquito species will also target mammals, spreading diseases such as lymphatic filariasis, for which there are 50 million human cases worldwide despite great efforts with mass drug administration programs.

Since the southern house mosquito's introduction to the U.S. in 1999, there have also been 48,000 reported human cases of West Nile virus (WNV).

Current control strategies for the southern house mosquito are heavily dependent on the use of insecticides. However, these impose hazards to both human and ecosystem health and are becoming increasingly ineffective with the rise of resistance.

Using species-specific genome editing

Advances in <u>genome editing</u> have allowed the development of genetic insect control methods, which could be highly effective and are species-specific.

The results have been published in *Scientific Reports*. Scientists showed that a method involving a gene editing tool called CRISPR/Cas9 could be used to successfully introduce a gene for a <u>fluorescent protein</u> into the genome of southern house <u>mosquitoes</u>. The gene could be passed on to



the next generation through mating.

This is a vital component of generating genetic pest management tools. It will allow the desired traits (such as the inability to spread a disease or produce fertile offspring) to be spread throughout a population.

The inserted gene produces red fluorescence proteins so that mosquitoes with one or more edited gene fluoresce red.

Scientists targeted an eye color gene for the insertion site of the fluorescence gene so mosquitoes that inherited two edited genes from their parents would have white eyes, not black. Both these traits make it easier for scientists to easily identify mosquitoes whose genomes had been modified.

Interestingly, mosquitoes that had <u>white eyes</u> (which had inherited two copies of the edited gene) did not survive into adulthood.

This effect is not seen when the same method has been applied in other species and highlights differences between mosquito genomes.

Although this specific eye color gene would not be targeted for creating any functional genetic pest management tools, it has provided proof that the method can work in this species.

A new avenue for pest management strategies

Professor Luke Alphey, Head of the Arthropod Genetics group at Pirbright said: "Other methods for genetically engineering the southern house mosquito have been attempted, but none of them work well enough to be useful in the field.

"This CRISPR/Cas9 method overcomes previous difficulties and opens



up a whole new avenue for scientists to generate genetic pest management strategies.

"This could ultimately prevent millions of people and animals from falling ill because of this invasive mosquito species."

More information: Deepak-Kumar Purusothaman et al, CRISPR/Cas-9 mediated knock-in by homology dependent repair in the West Nile Virus vector Culex quinquefasciatus Say, *Scientific Reports* (2021). DOI: 10.1038/s41598-021-94065-z

Provided by Medical Research Council

Citation: Scientists use gene editing tool to target mosquito-spread disease (2021, September 13) retrieved 26 April 2024 from <u>https://phys.org/news/2021-09-scientists-gene-tool-mosquito-spread-disease.html</u>

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