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Male Determining Gene Nix May Bring an End to Transmission of Diseases through Mosquitoes

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Male determining gene *Nix* may bring an end to transmission of diseases through mosquitoes

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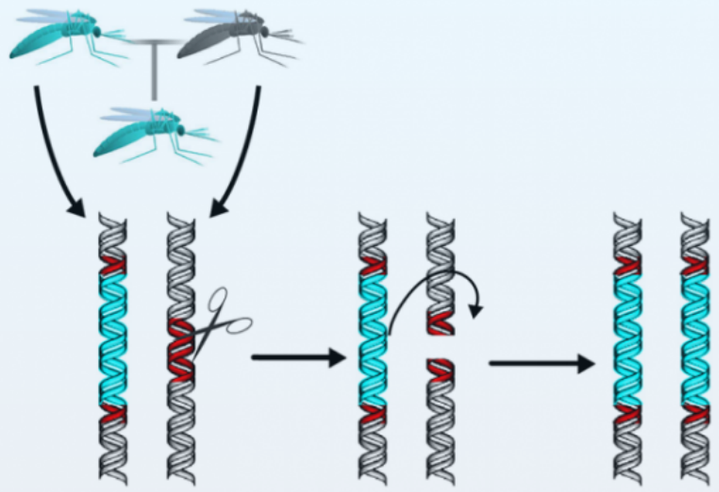
Abstract

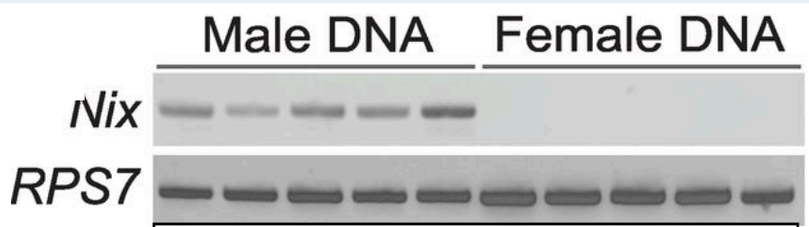
Female mosquitoes take blood from humans to gain protein needed to produce eggs, and in the process, they can transmit viruses such as Zika, Dengue, and chikungunya, or parasites, such as malaria. Male mosquitoes do not transmit diseases. This literature review will examine what is known about how sex is determined in mosquitoes and how this can be used to manipulate the females that spread diseases to humans. Using CRISPR Cas9, the lab of Zhijian Tu removed the Nix gene from mosquito embryos; all of these were feminized. Ectopic over-expression of Nix gene product in mosquito embryos caused even genetically female mosquitoes to have male genitalia. Nix has been shown to be a regulating factor for doublesex and fruitless, two other genes known to be involved in sex determination. The Nix gene has highly repetitive features similar to other genes in the Y chromosome of other organisms. Researchers in London have already demonstrated that CRISPR technology can be used to disrupt the female form of doublesex in a population of caged mosquitoes. The females become sterile and the population declines, Nix could be used in a similar way to change female mosquitoes to harmless males.

The figure above shows the male and female of *Aedes aegypti* mosquito. The female on the left sucks blood while the male on the right don't. *Aedes aegypti* was chosen for this research since it's a common vector for Zika, dengue, and chikungunya.

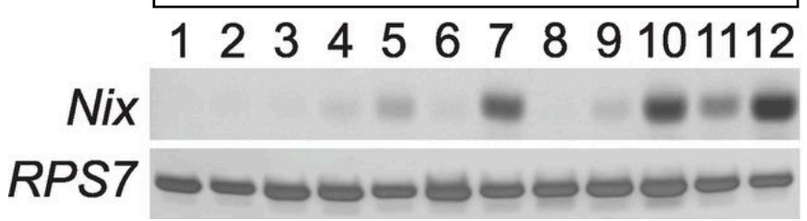


Cas9 is used as a molecular scissor in the process of gene drive to cut specific areas and cause a DNA double strand to break. This is exactly what scientists have been trying to do in the lab in London, where they have been working on altering genes similar to the sex determining gene Nix, in *Aedes aegypti*. The scientists in London were successfully able to use CRISPR cas9 in changing a gene known for regulating sexual development called doublesex (dsx) and found in *Anopheles gambiae.* The alteration caused in this gene led to deformed reproductive organs in females and caused some female to have mouth parts that are similar to male mouth in that they can't bite humans to get blood.

First, DNA strands of both males and female were aligned and compared to one another using the Illumina data of both sexes to look for a Y chromosome-like region called the M locus region that is only found in male mosquitos. The 164 contigs were aligned with RNA-seq data. RNA-seq is sequencing of cDNA made from mRNA using reverse transcriptase. Presence in the embryo RNA-seq and, absence from female-derived RNA-seq reduce the number of contigs by 140. From the 24 remaining strands, one had a sequence gene contig similar to the TRA2 gene, that is involved in the splicing of doublesex (dsx) and fruitless (fru). They named that gene Nix.



PCR was used to show that Nix is present in only male DNA and not on the female DNA. RPS7 is used as a control.



Reverse transcriptase PCR shows that Nix transcript appears in early embryonic stages, after 3-4 hours. RPS7 is used as a control.

Citation

Hall, Brantley, et al. "A Male-Determining Factor in the Mosquito *Aedes Aegypti*." Science, American Association for the Advancement of Science, 12 June 2015,
Stein, Rob. "Scientists Release Controversial Genetically Modified Mosquitoes In High-Security Lab." NPR, NPR, 20 Feb. 2019,

Acknowledgment

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